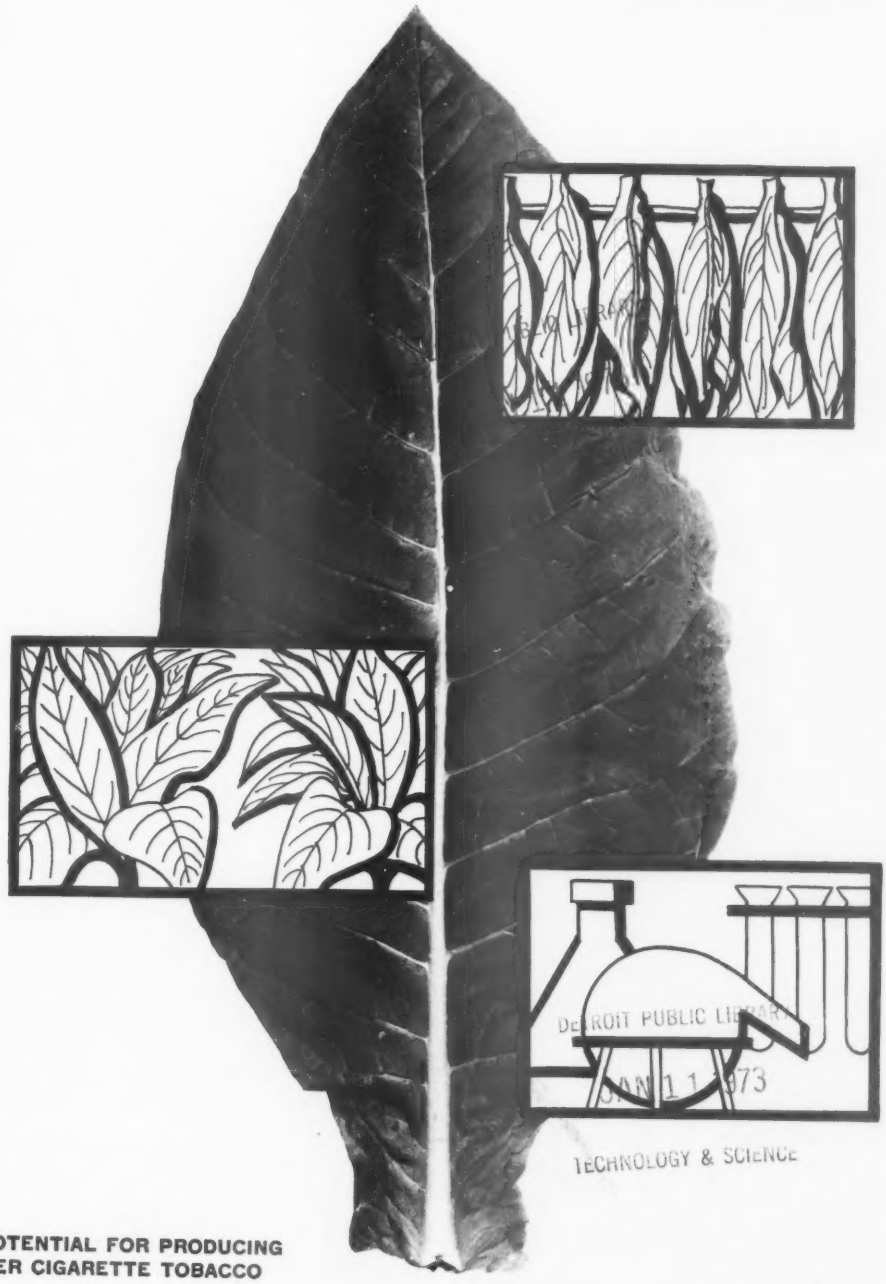
A2

AGRICULTURAL

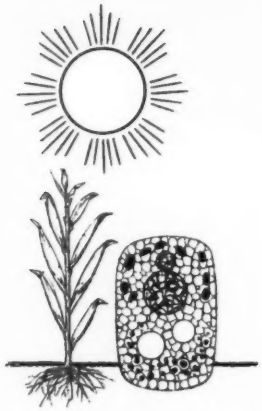
Science Review

COOPERATIVE STATE RESEARCH SERVICE U.S. DEPARTMENT OF AGRICULTURE VOL. 10, NO. 3



DETROIT PUBLIC LIBRARY
JAN 11 1973
TECHNOLOGY & SCIENCE

THE POTENTIAL FOR PRODUCING SAFER CIGARETTE TOBACCO



AGRICULTURAL SCIENCE REVIEW

Third Quarter 1972 Vol. 10 No. 3

CONTENTS

1 THE POTENTIAL FOR PRODUCING SAFER CIGARETTE TOBACCO

11 EVOLUTION OF HYBRID ALFALFA

19 PCB RESIDUES: THEIR SIGNIFICANCE TO ANIMAL AGRICULTURE

25 BREEDING SLASH PINE IN FLORIDA: A SUCCESS STORY

33 SCIENCE NOTES

35 FORUM

36 AUTHORS

AGRICULTURAL SCIENCE REVIEW is published quarterly by the Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C. 20250.

ROY L. LOVVORN, Administrator

EDITORIAL BOARD

J. G. HORSFALL, Connecticut Agricultural Experiment Station; D. W. THORNE, Vice-President for Research, Utah State University; T. W. EDMINISTER, Administrator, Agricultural Research Service; R. K. ARNOLD, Deputy Chief, Forest Service; R. G. GARNER and B. F. BEACHER, Cooperative State Research Service.

EDITORIAL CONSULTANTS

JOHN F. FULKERSON, BOYD W. POST, and ROLAND R. ROBINSON, Cooperative State Research Service.

EDITOR: JOEL W. WHEELER

Information in this magazine is not copyrighted and may be reprinted without permission. Mention of the source will be appreciated but is not required.

Solzhenitsyn on Scientists

Although Alexander Solzhenitsyn was barred by his government from receiving the 1970 Nobel Prize for literature, his would-have-been acceptance speech was published in August 1972 in the Nobel Foundation's yearbook.

In the speech, the Russian writer declares that "the timid civilized world has found nothing to oppose the onslaught of a sudden revival of barefaced barbarity, other than concessions and smiles."

With a tone of sorrow he sees the United Nations as having betrayed those who placed such high hopes in it. In the next breath he says: "It would seem that the appearance of the contemporary world rests solely in the hands of the scientists: All mankind's technical steps are determined by them. It would seem that it is precisely on the international goodwill of scientists, and not of politicians, that the direction of the world would depend. All the more so since the example of the few shows how much could be achieved were they all to pull together. But no: Scientists have not manifested any clear attempt to become an active force of mankind. They spend entire congresses in renouncing the suffering of others: Better to stay safely within the precincts of science. That same spirit of Munich has spread above them its enfeebling wings."

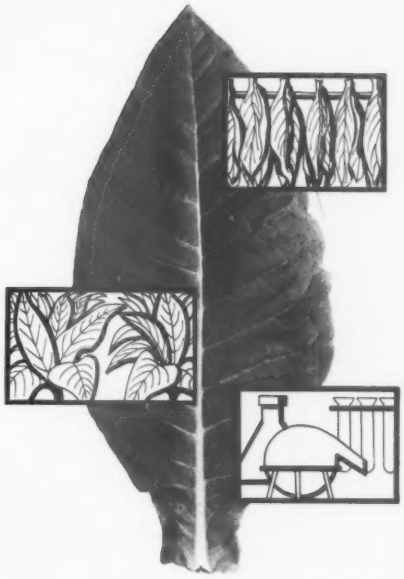
Few scientists are so arrogant as to believe that science can solve all the world's burning problems and fewer still would want the position and power to direct their solution. Most do want to help. Many are clearly trying hard.

It is in their "pulling together" not just with each other but with others that scientists can help most. Those others include politicians, humanists, philosophers, and—of a certainty—writers who, as Mr. Solzhenitsyn fervently believes, have it in their power "to help mankind, in its troubled hours, to see itself as it really is . . ."

World literature has a powerful helper in science.

To see something "as it really is." Isn't that what science is all about?—J.W.W.

The Potential for Producing SAFER CIGARETTE TOBACCO



T. C. TSO

IN 1964 the Surgeon General of the U.S. Public Health Service released a report prepared by his advisory committee on "Smoking and Health" (*27*). This report stated that "cigarette smoking is a health hazard of sufficient importance in the United States to warrant appropriate remedial action." Subsequent reports of similar nature have added to the complexity of problems of production and use of tobacco.

The U.S. tobacco industry ranks fourth in cash receipts among farm crops, and also fourth among farm crops in export value. It provides an income of $1.4 billion for 625,000 farm families, and more than $4 billion in tax revenue for Federal, State, and local governments. In 1970, 538 billion cigarettes were consumed in the United States. However, the per capita consumption in recent years has trended downward from 4,345 in 1963 to 4,000 in 1970 (*26*).

Many people will continue to smoke, and farmers will continue to grow tobacco as long as there is a market. It is necessary, therefore, to eliminate or reduce any potential hazards in cigarette smoke. The Agricultural Research Service (ARS) of the U.S. Department of Agriculture has underway inhouse and extramural research programs which include genetics, physiology, and biochemistry of the tobacco plant, and the chemistry and bioassay of tobacco smoke to help meet this goal. This research is coordinated with other tobacco and health-related research in the United States and around the world. Scientists from tobacco-producing States contributed significantly in this cooperative research effort by producing experimental tobaccos, supplying test materials, determining leaf composition, and taking part in special studies. For example, both the University of Kentucky and the North Carolina State University have an extensive tobacco research program relating to production, utilization, and marketing; some of these projects are supported by Federal funding with special interest in health-related tobacco problems. Scientists from Georgia, Tennessee, Maryland, and South Carolina collaborated with ARS in providing special samples as early as 1965. Other tobacco-producing States and tobacco industry also collaborated in many areas of health-oriented projects.

The primary objective of the health-related to-

bacco production program is to produce a good-quality, safer cigarette tobacco. This article discusses the potential and progress of this program.

Health-related Tobacco Research

TOBACCO differs from other major crops in that it is used primarily for pleasure and as a product of combusion. In the past, the quality of a leaf tobacco was judged by its smoke characteristics. In health-related tobacco research, leaf tobacco will additionally be evaluated by the relative biological activity of the smoke condensate.

Agricultural scientists have developed many plants for increasing yield, improving quality, or resistance to disease. The desired plants are selected by screening or comparison with standard varieties. The problem of tobacco smoking and health, however, adds a new dimension. First, there are at least 1,300 compounds which have so far been identified in tobacco and tobacco smoke. Only very limited information is available indicating the undesirability of a few of these components. Second, there is a lack of dependable systems for evaluating the "safety" of tobacco or tobacco products. Furthermore, cigarette smoke is considered to be a very "mild" carcinogen; the amount of undesirable component (or a combination of components) is minute and may easily escape detection.

The characteristics of cigarette smoke are functions of the physical and chemical properties of leaf tobacco which make the cigarette. Smoke constituents may be modified by changing leaf characteristics. The questions are: What kinds of changes are needed? and, how can these changes be achieved?

What is a Safer Cigarette Tobacco?

IN dealing with the problem of tobacco smoking and health, there are numerous variables involved, including leaf tobacco, cigarettes, smoking condition, and, most important, the smoker himself. One can only use the word "safer" in a relative sense under defined conditions based on available knowledge. A "safer" tobacco would generate cigarette smoke that is low in, or free from, known hazardous components and the smoke condensate would be low in, or free from, adverse activity in a biological test system. The principal tasks would be to define the characteristics of a safer cigarette and to develop means for evaluating the degree of "safety" before production of a safer cigarette tobacco.

Approaches to Developing Such Tobacco

THE following approaches are used in obtaining basic information for the development of a safer tobacco through production:

- Isolate and identify leaf characteristics which may be associated with undesirable smoke constituents or adverse biological activity.
- Determine the range of genetical, physiological, and chemical variabilities of available germ plasm affecting those characteristics.
- Regulate the biochemical process during senescence and curing.
- Compare the differences in smoke constituents and biological activities of major tobacco types varying in principal chemical components or cultural practices.
- Improve current production and curing practices for elimination or reduction of undesirable materials.

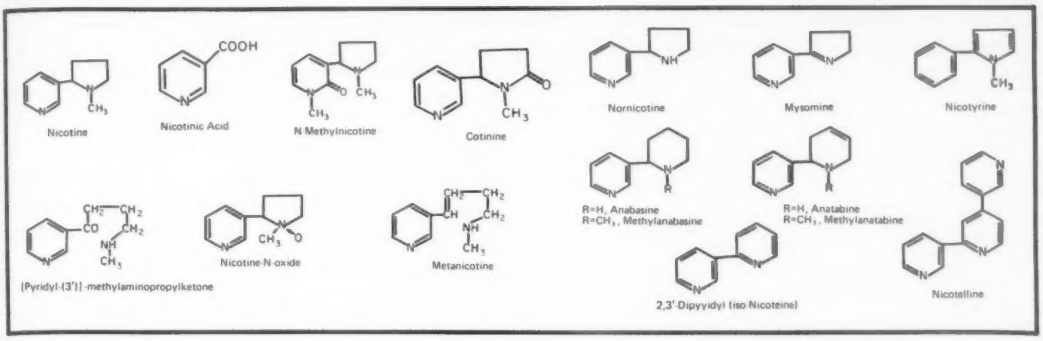
These approaches are attainable based on our current knowledge of the physiology and biochemistry of tobacco plants.

THEORETICAL AND PRACTICAL BASIS

Genetics and Germplasm

THE 63 species related to cultivated tobacco, *Nicotiana tabacum L.*, represent sources of divergent germplasm for biochemical and physical variations. These species differ widely in growth habit and in chromosome numbers. Breeding of new varieties has been successful via interspecific hybridization. Utilization of interspecific hybridization may induce extensive alterations in the chemical composition of the plant for health-oriented purposes. It should be noted, however, that only 25 to 30 *Nicotiana* species have been successfully crossed with *N. tabacum*, and interspecific hybridization and gene transfer is a time-consuming process which may require many manipulations. Nevertheless, much progress has been made in recent years in utilizing this wide range of germplasm.

Within the species of *N. tabacum*, many varieties and types show wide differences in chemical composition. A large collection of materials is available to the USDA, including about 1,000 tobacco introductions and 400 established varieties. Preliminary examination among samples from tobacco introductions showed wide variation in many major



Nicotine and relatives.

organic components. The range of variability for selected characteristics in progeny with *N. tabacum* crosses is probably not as wide as that found in progeny produced from hybridization with *Nicotiana* species.

In addition to breeding through selection or hybridization, parasexual interspecific hybridization by protoplast fusion and regeneration has been demonstrated (*6*). This new procedure would indeed lead to great opportunities for developing a safer tobacco. Induction of beneficial mutations by chemical or physical means are also feasible.

Dynamic Balance During Growth

SMOKE constitution is greatly affected by the balance between carbon and nitrogenous fractions of leaf tobacco (*18*). To interpret the phytochemistry of tobacco, it is desirable to consider such changes based on the Krebs tricarboxylic acid (TCA) cycle. Carbon dioxide from air is assimilated by the tobacco leaf through photosynthesis. Inorganic nitrogen is assimilated by roots from the soil. Hydrogen, which is liberated by dehydrogenation from the components of the TCA cycle, is used to reduce nitrate to ammonia which in turn is utilized by members of the TCA cycle (oxaloacetic acid, α-ketoglutaric acid) to form aspartic and glutamic acids. Ammonia is also taken up by pyruvic acid to form alanine.

These products serve as nitrogen sources in the formation of other amino acids. The net result of nitrogen assimilation is the utilization of a portion of newly photosynthesized carbon chains into the nitrogenous pool. When the nitrogen supply is abundant there will be more synthesis of amino acids and nicotine and less sugars and starch. If the nitrogen supply is limited, there will be an excess accumulation of acetate in the TCA cycle which results in higher production of carbohydrates, fats, volatile oils, resins, and polyterpenes. These variations are also reflected in the leaf texture, color, porosity, and combustibility.

Thus, leaf characteristics are controlled by changes in the environmental conditions. Some excellent examples are oriental, bright, and cigar tobacco types. The oriental type tobacco is produced with limited supply of nutrients and water, and therefore the balance is toward carbohydrates and the leaves are highly aromatic. Cigar tobacco represents the other extreme and is grown with an abundant nitrogen supply. Protein and nicotine contents are high. Bright or flue-cured tobacco may be considered intermediate, but slightly toward the carbon side; it is grown with a moderate supply of nitrogen which is depleted when the plant reaches maturity.

Other factors such as the height of the tobacco plant at the time of decapitation, light intensity, and temperature all exercise essential effects on this dynamic balance. If opping is very low, with only a small number of leaves remaining on a plant, even limited amounts of available nitrogen may shift the balance toward the protein-nicotine side. Under low light intensity and low temperature conditions tobacco leaf can hardly be aromatic even when the nitrogen supply is very low.

Metabolism During Senescence and Curing

SENESCENCE is the phase of plant growth that extends from maturity to actual death, and is characterized by an accumulation of metabolic products, increase in respiratory rate, and a loss of dry matter (*20*). At the senescence stage, enzyme activities (especially hydrolytic and other degradative enzyme systems) are intensified. These systems are responsible for breakdown of functional and structural components of the cell, such as proteins, nucleic acids, carbohydrates, and lipids. The latter stage of senescence resembles the early stage of leaf curing. Curing is a vital process which falls into the category of starvation phenomena or inanition of excised plant parts.

The most conspicuous chemical conversions during curing involve two phases. The first phase is dominated by hydrolytic enzymes and occurs in either flue-curing or air-curing. In this phase, disaccharides and polysaccharides are hydrolyzed to simple sugars; proteins are hydrolyzed to amino acids which undergo oxidative deamination; and the pectins and pentosans are hydrolyzed to pectic acid, uronic acid, and methyl alcohol. The second phase is dominated by oxidative reactions and takes place mostly in air-curing processes. Among the conversions are the following: oxidation of simple sugars to acids, O_2 and H_2O; the increased oxidative deamination of amino acids leads to the formation of ammonia and amides, particularly asparagine; changes in organic acids, including conversion of malic to citric acid and also decarboxylations; and the oxidative and polymerization of phenols to brown products. There is a small decrease in alkaloids and some loss of dry weight.

Metabolic changes as described above can be regulated toward a desired direction.

Smoke Composition and Biological Activity

CIGARETTES made from tobacco varying in farm production practices delivered various amounts of nicotine and total particulate matter (TPM) in the smoke (*17*). The nicotine content in tobacco smoke seems largely dependent on nicotine content of leaf tobacco, but TPM of the smoke seems to be more related to the amount of tobacco required to make the cigarette, as well as the density and total number of puffs. Adjustment can be made in breeding and in farm production practices to modify nicotine content of tobacco smoke. The TPM content can also be modified, but probably to a lesser degree.

Leaf composition differs from crop to crop. Even within the same plant, leaves from various positions on the stalk vary considerably in their physical properties and chemical composition (*20*). Leaves from the lower positions deliver less TPM in smoke than those from middle and upper positions (*28*). However, leaves from upper and middle positions are more aromatic than those from lower positions.

Employing mouse skin tests for tumorigenicity of smoke condensate, tobacco leaf and tobacco midrib were compared for their relative biological activity (*15, 28*). The specific tumorigenicity of condensate from cigarettes made of leaf was higher than that of condensate from cigarettes made from midrib. The specific activity of condensate from cigarettes containing a mixture of leaf and midrib had an intermediate value. The tumorigenicity ratios were, leaf 1.12, mixture of leaf and midrib (in 7:3 ratio) 1.00; midrib 0.53 (*15*). In addition, smoke condensate from cigarettes made from reconstituted tobacco sheet had a lower specific tumorigenicity than smoke condensate from cigarettes made from the same blend of flue-cured tobacco processed in the conventional manner. The tumorigenicity ratios were standard blend tobacco, 1.00, reconstituted sheet tobacco 0.62 (*15*).

The above-mentioned theoretical and experimental basis indicates that plant material, smoke composition, and biological activity are closely interrelated. Properties of leaf material can be modified through genetical and biochemical manipulation—from seed to curing. It is true that one cannot use only leaves from certain stalk positions and discard the rest, but opportunity exists that one can "remold" the characteristics of leaf material to meet required conditions even after harvesting.

EXPERIMENTAL PROGRESS

Leaf, Smoke, and Biological Activity

EXPERIMENTAL leaf samples were produced and special cigarettes were made for this study. One group of samples involved only bright type tobacco; the other group involved blend leaves of bright, burley, Maryland, and oriental tobacco types (*23*). Each sample of leaf tobacco was characterized in detail according to the botanical, physical, and chemical properties as follows; trichome

number; leaf thickness; fire-holding capacity; color; moisture equilibrium; specific volume; pH; various inorganic elements; carbohydrates (individual sugars, starch, and cellulose); nitrogenous fractions (individual alkaloids, volatile bases, total N, ammonia N, nitrate N, α-amino N, secondary amines and individual amino acids); phenolic compounds (chlorogenic acid, rutin, scopoletin, scopolin, quinic acid, and lignin); organic acids (succinic, oxalic, malic, and citric); fatty compounds (petroleum ether extracts, total lipids, fatty acids of various chain lengths); phytosterols (cholesterol, campesterol, stigmasterol, and β-sitosterol); agricultural chemicals (DDE, DDT, TDEE); organoleptic characteristics (aroma, flavor, strength and quality); and yield.

The experimental cigarettes were also characterized by weight, static burning rate, pressure drop, average puff number, smoke delivery (H_2O, TPM, and nicotine), and smoke chemistry including phenols, cresols, acetaldehyde, acrolein, HCN, benzo(a)pyrene (BaP), benz(a)anthracene (BaA), smoke pH, CO, CO_2, and N-nitrosamines. Bioassay of smoke condensate from each sample was conducted either by long-term mouse skin painting or by short-term tests such as sebaceous glands, cell culture (virus-transformed and chemically transformed cells), sarcoma virus in quail or in mice, alveolar macrophage, cell toxicity (Mammalian cells) ciliary transport activity, and E. *coli* system.

Approximately 20,000 pieces of information from each test will be obtained for computerization. From this mass of data, precursor and product relationship among many variables can be established. Such relationships, as indicated from preliminary results, vary with tobacco types, and with the blending formulation of the cigarettes. Furthermore, the TPM and BaP content in the smoke of a cigarette can be predicted when the plant variety and leaf stalk positions are known. Variety itself reflects genetical characteristics, while stalk position indicates differences in botanical, physical, and chemical variability. The effect of multiple variables can also be predicted by multiple regression.

Genetical and Biochemical Aspects

CURRENT knowledge of the inheritance of alkaloids in the tobacco plant is well advanced. It is possible to breed tobacco varieties with a wide range of nicotine content. Next to nicotine, among the most commonly found alkaloids in tobacco are nornicotine, anatabine, and anabasine. More than 10 other pyridyl compounds have also been detected. The genetics of conversion between nicotine and nornicotine has been extensively studied, and it is known to be controlled by a single dominant gene. New strains of tobacco plants with extremely low levels of nicotine are already being developed (*11*). In Burley tobacco, a low alkaloid (LA) strain of Burley 21 has a nicotine content of approximately 0.2 percent (*12*). In bright type tobacco, a low alkaloid breeding line containing 0.2 percent nicotine is being developed.

A threefold variation of total phytosterol content was found among common bright tobacco varieties. The results also suggested the possibility of obtaining low sterol varieties through breeding without affecting yield or alkaloid content (*7*). The amount and distribution of these phytosterols in the tobacco plant is affected by tobacco types, varieties, stage of development, method of curing, and many other environmental and cultural factors (*8, 9*). Sterols may take part in the metabolic changes of the plant. Interconversion among sterols in tobacco plants has been observed.

Preliminary studies of polyphenols in tobacco suggest that levels of this group of compounds are controlled by multiple genes. Phenolic compounds are known to affect leaf color, quality, and smoke strength. Rutin and chlorogenic acids are most abundant, while scopolin, scopoletin, quinic acid, and related compounds commonly occur. Five-fold differences of phenolic compounds were observed in some randomly selected samples. Tobacco type, variety, stalk position, maturity, light, temperature, nutrient, and curing were reported to affect the composition and levels of phenolic compounds in tobacco. Two aromatic amino acids, phenylalanine and tyrosine, are major precursors of phenolic compounds in tobacco plants. Progress has been made in determining the enzyme systems involved and means of their regulation (*1, 13*).

Lipids, both saponifiable and unsaponifiable, are present in tobacco. Cigarette tobaccos differ in the amount and distribution of lower fatty acids as well as higher ones. Fatty alcohols are also present. A constant change in the level and composition of

fatty acids and crude lipids in tobacco plants occurs during growth, senescence, and curing. Higher fatty acids, especially unsaturated ones, decrease beginning at the early senescence. The most extensive decrease occurs at the yellowing stage of leaf curing. Such a decrease is approximately in proportion to the degree of fatty acid unsaturation. During the same period, there is a slight increase of comparatively shorter and more saturated fatty acids (*22*).

Indications are that genetical and biochemical manipulations can be employed to induce changes in leaf composition and thus alter the smoke constituents toward a desired direction. However, one needs to recognize the complexity of the problem, especially the interaction among various biochemistry processes in plant metabolism. Progress can be achieved only after we acquire a better understanding of basic knowledge on the tobacco plant.

Agronomic and Cultural Aspects

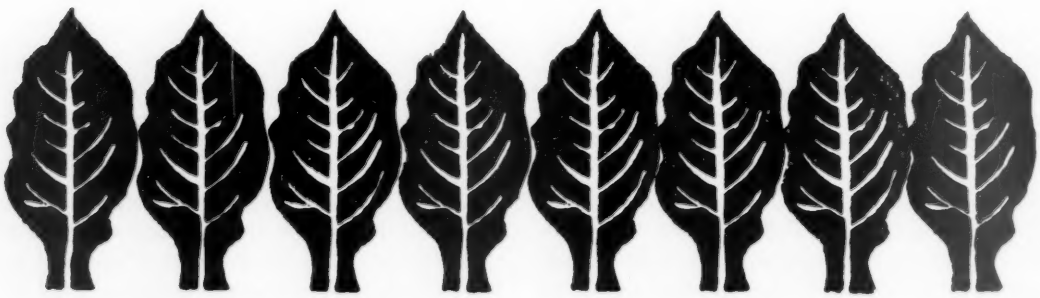
IT is sometimes suggested that the tobacco and health issue, as a more recent problem, is possibly related to changes in varieties and modernized production practices. Such a hypothesis is based on the fact that varieties in use for many decades have been replaced with new high-yielding, disease resistant ones, new curing methods are used for convenience and to save labor, various fertilizer formulations, pesticides, and suckering chemicals are used to increase yield and to reduce the cost of production. Many studies are underway to evaluate these factors. Experimental tobaccos have been made into cigarettes and smoke composition and bioassay studies are being conducted.

The broad scope of these investigations is illustrated by the following studies:

- An old variety (Virginia Bright Leaf) versus a new variety (Coker 319) together with an old flue-curing method versus a new open-flame curing method.
- Bright type tobacco which was air-cured versus air-cured type tobacco which was flue-cured.
- Burley type tobacco produced with 300 lb. nitrogen per acre in addition to vetch cover crop versus no nitrogen after rye.
- Tobacco mosaic virus (TMV) infested leaf versus TMV free leaf.
- Leaves of three *Nicotiana* species.
- Maryland type tobacco varying in major alkaloid (nicotine versus nornicotine) and in rate of nitrogen fertilization (16 lb. versus 150 lb. N/A).
- Tobacco produced with various nematicides and soil fungicide (DD, soilbrom 85, Mocap, Vorlex, telone).
- Bright tobacco with high and low alkaloid levels.
- Tobacco treated with various suckering chemicals (maleic hydrazide, Penar, methyl caprate, 1-octanol, and 1-decanol).
- Various post-harvest and post-curing treatments. In addition, pure chemicals whether used as pesticides or growth regulators are being tested for their relative biological activity.

Effects of nitrogen fertilization and various nitrogenous fractions in tobacco were extensively studied (*2, 5*). A number of these studies were conducted in cooperation with State tobacco scientists.

While we are searching for answers to the above-mentioned agronomic and cultural variables, we are, at the same time, conducting studies on problems of basic interest and immediate concern. In this area, five important tobacco-health studies



can be noted: Mycotoxins, radioelements, suckering agents, N-nitrosamines, and insect resistance.

Tobacco plants come into contact with various organisms at all stages of handling. Among the many toxins produced by microorganisms, aflatoxin from *Aspergillus flavus* is the most potent carcinogen. *A. flavus* was reported to be present on tobacco leaf but aflatoxins were not detected in an examination of hundreds of leaf samples from different sources under mold or nonmold conditions. This result is by no means a positive insurance that aflatoxins would never occur on any tobacco. Several tests were made by "spiking" up to 5,000 p.p.b. of aflatoxin B_1 onto cigarette tobacco. These cigarettes were smoked and smoke condensate was collected to determine aflatoxin recovery. Results showed that aflatoxin B_1 was either totally decomposed or transformed to other products during the process of combustion (*24*). This study eliminates aflatoxin as one of the most urgent concerns of the tobacco-health problem.

Naturally occurring radioelements in manufactured tobacco and in smoke have been investigated many times. Earlier reports concern mostly beta activity of K–40, while recent studies mainly refer to alpha activity, especially of the radium and thorium series. The problem of polonium-210 is of special interest, for this element is volatile at the combustion temperature of manufactured tobacco. Po–210 in tobacco plants is derived from either the soil or the air. It may be taken up directly from the soil or may result from radioactive decay of lead-210 or radium-226 taken up from the soil. It may also result from radioactive decay of the daughters of radon-222 deposited on the leaves. In attempting to establish its origin, we studied the natural radioactivity in different types of leaf tobacco of various years and localities; in various tobacco soils and in various tobacco fertilizers.

Other Studies

Experiments were also conducted by supplying Rn–222, Po–210, and Pb–210 to growing tobacco plants. Results showed that the main source is from tobacco root absorption, and that phosphorus fertilizer is the principal carrier of Ra–226 and its daughters. Po–210 and Pb–210 in the soil can also be removed to a certain extent (*19*). However, most recent studies appear to show that the level of Po–210 in leaf tobacco, and thus in cigarette smoke, is so low that it is not of a significant health concern. If further studies indicate the need, fertilizer can be purified free from the source of Po–210 for tobacco production.

Maleic hydrazide (MH) has been used as a tobacco suckering agent for nearly 20 years. In the early 1960's there were questions concerning the desirability of MH with regard to cigarette leaf quality. In recent years, there have been contradictory reports on the possible effects of MH on tobacco smoking and health. During the past decade considerable research effort has been devoted to searching for a substitute for MH. One group of compounds, fatty acid derivatives, was found to be effective in controlling tobacco sucker growth by contact action (*14, 16*), and results from cigarette smoke and bioassay data are very promising (*21*). Comparatively, the systemic compound, MH, is much easier to use by farmers.

Some N-nitrosamines are known as carcinogens. The process of metabolic activation and possible detoxification in biological system of these compounds is still under study. Earlier reports indicated the possible presence of volatile nitrosamines in tobacco smoke. This possibility received much attention recently because published data indicated that the addition of nitrate salts in tobacco may reduce the yield of BaP and other polynuclear aromatic hydrocarbons (PAH) in cigarette smoke (*3, 10*). If one increases the amount of nitrogen fertilization in field tobacco production, it would result in an increase in the level of nitrate in leaf tobacco, but at the same time it may also increase the risk of N-nitrosamines in smoke.

To examine this problem, experimental tobaccos were produced for cigarette smoke evaluation. This study is being conducted in collaboration with four domestic and foreign laboratories. Available data appears to vary widely among laboratories. Generally, results show that experimental leaf tobacco produced with extremely high levels of nitrogen fertilization (2 or 3 times the normal level) lead to the presence of dimethylnitrosamine (DMNA) in cigarette smoke (results vary from 1 to 32, trace to 50, and 27 to 140 ng/cig. from three different laboratories). However, DMNA was found at marginal level of detection (below 10 ng/cig.) or was not detected at all in smoke from cigarettes of experimen-

479-943 O - 72 - 2

tal leaf samples produced under normal or low nitrogen fertilization levels.

By regulating nitrogen metabolism in plants through production processes, plant scientists can reduce the level of volatile bases in the leaf and thus nitrosamine in the smoke. Several studies are in progress to examine multiple factors in leaf which may affect nitrosamines in smoke. Other industrial techniques, such as filtration, may also remove, to a large extent, nitrosamies in tobacco smoke.

A study of both academic and practical importance, is the search for tobacco plants which are disease and insect resistant in order to eliminate pesticide problems. Breeding for disease resistance has been practiced for many years, but breeding for insect resistance is relatively new. Large numbers of *Nicotiana* species were tested for their resistance to feeding damage by tobacco hornworm and budworms (*4*). Results show that plants with high resistance to one insect species would sustain considerable damage of the other. Most *Nicotiana* species which are resistant to larvae of the hornworm or budworm tend to show close phylogenetic relations. Thus, resistance to the tobacco budworm was most often found among members of the subgeneric section *Alatae*, and resistance to the hornworm was found among species of section *Repandae* and *Bigelovianae*. Progress made so far indicates that insect resistance can be achieved in tobacco but it will be a costly and time-consuming project.

Novel Approach in Leaf Curing

LEAF tobacco, as a natural product, follows the laws of nature in all aspects of growth, development, and biochemical changes. Certain organic components are primary products and some are secondary in nature. Their presence is the result of essential biochemical processes of life. One cannot by breeding or culture eliminate from leaf tobacco certain essential products which may not be desirable in smoking and health. Such products, however, may be reduced or eliminated after harvesting. A new method is being developed for this purpose through leaf "curing" (*25*).

In the conventional tobacco curing processes, harvested tobacco is cured as the intact individual leaf or as leaves attached onto stalks. There is little opportunity to improve the leaf quality further than the conditions which a harvested fresh leaf provides. In other words, once the leaf is ready for conventional curing, conditions are "set" for what one may expect as the maximum leaf quality. The alternative is, therefore, a new approarch for leaf-curing based on the understanding and regulation of the biochemical processes involved. Such a regulation, however, can hardly be accomplished by curing the intact leaf under conventional methods. This new approach is to "cure" leaf material in a homogenized state. Experiments were conducted by homogenizing the harvested fresh leaf through mechanical chopping followed by physical, chemical, or biological treatment.

In efforts to improve this curing process, several experiments have been performed. These include various preharvest field treatment procedures, methods of homogenization, regulated environmental conditions, use of enzyme inhibitors and/or promoters, removal of undesired fractions, addition of desired compounds, and controlled dehydration and incubation. Such a "cured" tobacco mass is then ready for manufacture into sheets by methods available commercially. We find that this homogenized leaf curing (HLC) procedure saves up to almost 50 percent of labor in certain tobacco types, and offers vast opportunity for regulating the final composition of cured tobacco material. In addition, one can make tobacco sheets to meet required physical properties. Preliminary evaluation by smokers of the first group of experimental cigarettes made from this HLC process was quite satisfactory. This homogenized curing method opens a new, wide horizon for tobacco improvement, especially adaptable in health-related research.

A PERSPECTIVE OF THE POTENTIAL

IN dealing with an organic material as complex as tobacco, there are limitations in the range of possible changes. Of primary concern to us are the biological interaction and metabolic balance within a plant system, the combustion products and their biological activity. At the same time; the basic quality factors need to be incorporated into the "safer" leaf tobacco. Without desired quality for consumer acceptance, a "safer" tobacco means no tobacco. Our goal is to develop a tobacco which can satisfy farmers, manufacturers, and consumers in quality as well as in health needs.

The task of developing a safer tobacco will be

difficult and challenging, but the potential is great. Preliminary findings are encouraging. There are opportunities for genetical changes, and for improvements in cultural and curing practices. There is little doubt that a "safer" tobacco can be developed. However, much effort is needed in providing basic information as well as its practical application. We face many questions in health-related tobacco research, and their answers are not easy. Achievement can come only through a well supported, coordinated, and balanced multiple-discipline research program.

LITERATURE CITED

(1) Andersen, R. A., Chaplin, J. R., Currin, R. E., and Ford, Z. T.
1970. Plant phenols in flue-cured tobacco fertilized at different rates. Agron. Jour., 62:415–417.

(2) Atkinson, W. O., and Sims, J. L.
1971. Nitrogen composition of burley tobacco. II Influence of N fertilization, suckering practice and harvest date on yield, value, and distribution of dry matter among plant parts. Tobacco Sci., 15:63–66.

(3) Bentley, H. R., and Burgan, J. G.
1960. Polynuclear hydrocarbons in tobacco and tobacco smoke. Analyst 85:727–730.

(4) Burk, L. G., and Stewart, P. A.
1971. Survey of resistance among Nicotiana species to tobacco hornworm and budworm larvae. Tobacco Sci., 15:32–34.

(5) Bush, L. P., Sims, J. L., and Atkinson, W. O.
1969. Volatile nitrogenous bases and aliphatic secondary amines of burley tobacco. Can. Jour. Sci., 50:289–294.

(6) Carlson, P. S., Smith, H. H., and Dearing, R. D.
1972. Parasexual interspecific plant hybridization. Proc. Nat. Acad. Sci., USA 69:2292–2294.

(7) Cheng, A. L. S., Chaplin, J. F., and Tso, T. C.
1968. Sterol variation in flue-cured tobacco varieties. Tobacco Sci., 9:33–34.

(8) Davis, D. L., Legg, P. D., and Collins, G. B.
1970. Distribution of total 3-β-hydroxysterols in burley tobacco. Crop Sci., 10:545–547.

(9) Grunwald, C., Bush, L. P., and Keller, C. L.
1971. Variation in sterols, alkaloids and polyphenols of two Nicotiana varieties under different nitrogen fertilization and drying processes. Agr. and Food Chem. 19:216–221.

(10) Hoffmann, D., and Wynder, E. L.
1967. The reduction of the tumorigenicity of cigarette smoke condensate by addition of sodium nitrate to tobacco. Cancer Res., 27: 172–174.

(11) Legg, P. D., Chaplin, J. F., and Collins, G. B.
1969. Inheritance of percent total alkaloids in N. tabacum 1. Jour. of Heredity, 60:213–217.

(12) Legg, P. D., Collins, G. B., and Litton, C. C.
1970. Registration of 1A burley 21 tobacco germplasm. Crop Sci., 10:212.

(13) Sheen, S. J.
1970. Polyphenol content, polyphenoloxidase and peroxidase activity in certain Nicotiana species, varieties and interspecific hybrids. Theoretical and Applied Genetics, 40:45–49.

(14) Steffens, G. L., Tso, T. C., and Spaulding, D. W.
1967. Fatty alcohol inhibition of tobacco axillary and terminal bud growth. Agr. and Food Chem., 15:972–975.

(15) Tobacco Research Council
1970. Review of activities 1967–69, London.

(16) Tso, T. C.
1964. Plant growth inhibition by some fatty acids and their analogues. Nature 202:511–512.

(17) ———
1968. Effect of farm production practices on nicotine and total particulate matter in cigarette smoke. In "Toward a Less Harmful Cigarette", Nat'l Cancer Inst. Monograph 28, U.S. Dept. Health, Education and Welfare, Public Health Ser., Bethesda, Md.

(18) ———
1969. Tobacco. Encyclopedia of Chem. Tech. John Wiley and Sons, Inc., New York 20:504–525.

(19) ———
1970. LIMITED REMOVAL OF Po^{210} AND Pb^{210} FROM SOIL AND FERTILIZER BY LEACHING. Agron. Jour., 62:663–664.
(20) ———
1972. PHYSIOLOGY AND BIOCHEMISTRY OF TOBACCO PLANTS. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pa., U.S.A.
(21) ———
1972. MANIPULATION OF LEAF CHARACTERISTICS THROUGH PRODUCTION—THE ROLE OF AGRICULTURE IN HEALTH RELATED TOBACCO RESEARCH. A report presented at the Second International Conference on Smoking and Health, Jour., Nat'l Cancer Inst., (In press).
(22) Tso, T. C., AND CHU, H.
1970. EFFECT OF GROWTH, SENESCENCE AND FLUE-CURING ON FATTY ACID COMPOSITION OF TOBACCO. Agron. Jour., 62:512–514.
(23) Tso, T. C., AND GORI, G. B.
1972. EFFECT OF TOBACCO CHARACTERISTICS ON CIGARETTE SMOKE COMPOSITION. In "The Chemistry of Tobacco and Tobacco Smoke." Plenum Publ. Corp., New York.
(24) Tso, T. C., AND SOROKIN, T.
1967. EXAMINATION OF AFLATOXIN B_1 IN LEAF TOBACCO AND IN CIGARETTE SMOKE CONDENSATE. Beit. zur Tabakforschung, 4:18–20.
(25) Tso, T. C., LOWE, R., AND DEJONG, D. W.
1972. NOVEL APPROACH IN LEAF CURING. Unpub. progress rpt.
(26) U.S. DEPARTMENT OF AGRICULTURE
1971. AGRICULTURAL STATISTICS. Wash., D.C.
(27) U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, PUBLIC HEALTH SERVICE
1964. SMOKING AND HEALTH. Report of the Advisory Committee to the Surgeon General of the Public Health Service. Pub. No. 1103, Bethesda, Md.
(28) WYNDER, E. L., AND HOFFMANN, D.
1967. TOBACCO AND TOBACCO SMOKE. Studies in Experimental Carcinogenesis. Academic Press, New York.

TOBACCO AND HEALTH

TOBACCO had long been smoked by the West Indians who greeted Columbus as he was discovering America. Relics of our own mound builders indicate that pipe smoking was a very ancient custom. The Indians of Mexico and South America, who cultivated tobacco with great care and skill, believed it possessed enormous curative powers for bronchitis, rheumatism, and asthma.

For a half century after tobacco was introduced into Europe, generally credited to the Frenchman Jean Nicot in 1561, it was used almost exclusively for medicinal purposes and was considered to have wonderful curative properties.

During the first half of the 17th century, indulgence in tobacco became widespread in most of Europe. During that time some authorities vigorously opposed its use.

One of the first in a continuing line of reports contending that smoking causes disease was published in 1859. It was a report on 68 patients in a hospital in Montpellier, France, who had cancer of the lips, tongue, tonsils, and other parts of the mouth. Of that number, 66 smoked short-stemmed pipes.

EVOLUTION OF HYBRID ALFALFA

W. R. CHILDERS AND D. K. BARNES

ALFALFA is the most important forage legume grown in North America. The forage produced on the present 30 million acres of alfalfa is utilized as hay or silage in dairy and beef cattle feed or is dehydrated and used in rations for poultry and other domestic animals. Alfalfa is an important source of protein, carotene, minerals, and contains a rich supply of most essential vitamins. According to Stahmann (*16*), alfalfa produces a greater yield of proteins and essential amino acids per acre than any other agronomic crop. Methods of leaf-protein extraction from alfalfa are presently being implemented. Some scientists suggest that alfalfa proteins may one day be utilized for human consumption. In the future alfalfa should continue to deserve its title as "Queen of the Forages."

In areas where alfalfa is adapted, growers usually choose it over other forage crops because of its high yield potential. Forage yields of 8 to 10 tons of hay per acre have been reported from several Midwestern States and 10 to 12 tons per acre are routinely harvested in irrigated areas in the Southwestern United States. Even though high yields are possible the annual average yields in many States remain less than 3 tons per acre. Progress in increasing alfalfa yields over the period 1954–65 has been relatively good when compared to other forage crops, but when compared to field crops such as corn and sorghum these increases have been very modest (Table 1). It, therefore, is not surprising that alfalfa breeders have been seeking new ways to improve the yield potential of alfalfa varieties.

Nearly all present varieties of alfalfa have been developed as improved populations or as synthetic varieties (a variety produced by inter-pollinating a number of genotypes selected for good combining ability and subsequently maintaining the variety by open pollination). Forage yield has been an important consideration in varietal development. How-

TABLE 1.—*Changes in yield per harvested acre of selected crops*[1]

Crops	Unit	1954	1965	Percentage increase
Corn grain	Bushel	37.1	73.1	97
Sorghum grain	Bushel	19.0	50.1	164
Barley	Bushel	28.5	43.5	53
Soybeans	Bushel	20.1	24.4	21
Corn silage	Ton	7.5	10.6	41
Alfalfa, alfalfa mix	Ton	2.10	2.48	18
Clover, timothy mix	Ton	1.43	1.53	7

[1] From Address by A. A. Hanson (Agricultural Administrator, Crops Research Division, Agricultural Research Service, Beltsville, Md.) to the National Conference on Forage Quality Evaluation and Utilization, Sept. 2–5, 1969, Lincoln, Nebr.

ever, the necessity to develop varieties with improved pest resistance has been paramount in most breeding programs. New varieties have increased alfalfa yields, but this has been due in part to protection of the crop from periodic losses to pests. Some of the other increases in production during the last 10 years must be attributed to improved crop management practices such as high fertility and early harvesting. After considering the yield advances from pest resistance and improved crop management, it becomes apparent that the progress attributable to genetic factors, such as increased plant size, rate of plant growth and/or photosynthetic efficiency, has been limited.

Hybrids the Answer?

DURING the late 1960's hybrid alfalfas were described as having possibilities for improving crop production. A few breeders could foresee almost unlimited possibilities for the future of hybrid alfalfa. Other alfalfa breeders were less enthusiastic and raised questions about the economics of breeding, producing seed, and growing hybrid alfalfas as compared to synthetic varieties. Answers to these questions are slowly beginning to unfold because hybrids are now a reality, not just a futuristic possibility. Limited hybrid seed is being sold to growers in the Midwest. It is not yet possible to proclaim a final verdict about the success of hybrid alfalfa because its evolution is not yet complete. New technology is still unfolding to help breeders select better hybrid parents and to increase the efficiency of seed production. Much more long term performance data will be needed before hybrids and synthetics can be compared realistically.

This article was prepared because of the interest, glamour, and controversy that has been generated by hybrid alfalfa. We have attempted to present background information about the history of alfalfa hybrids, describe the present status of hybrid research, indicate problems that remain unsolved, and discuss the future outlook for alfalfa hybrids and their possible impact on agriculture.

The use and promotion of hybrids has appeal to plant breeders, seed producers, and growers; but often for different reasons. The plant breeder and the grower are anxious to use hybrids to capitalize on the fact that superior first-generation hybrids can surpass both parents in yield. This phenomenon has been termed hybrid vigor or heterosis. Hybrids present breeders with a tool that allows them to fix desirable traits into a variety easier than by use of the conventional synthetic variety approach. Hybrids also have the potential for increasing the ease and speed with which locally adapted varieties could be developed. Seed growers and commercial sales organizations are interested in hybrids for their potential high-yielding characteristics and because of the sales appeal of the term "hybrid." To most people familiar with agriculture the term is practically synonymous with the success story of hybrid corn. Hybrids also offer a means of variety protection to the developer and seed producer that is not

available with other types of variety synthesis. The performance of broad-based varieties can nearly be duplicated by simply producing seed of that variety for an additional generation. However, it is not possible to duplicate the performance of a specific hybrid combination without having the same parental stocks.

Until July 31, 1968, when Congress amended the Federal Seed Act Regulations, the term "hybrid" was defined primarily for use with corn and sorghum. Now the definition of hybrid can be applied to varieties of nearly all crops that meet the following requirements. Hybrid seed refers to the first generation of seed of a cross produced by controlling the pollination and by combining (1) two or more inbred lines; (2) one inbred line or a single cross with an open-pollinated variety; or (3) two selected clones, seed lines, varieties, or species. Controlling the pollination refers to any biological or physical method of hybridization that will produce at least 75 percent hybrid seed. If 95 percent or more of the seed is hybrid then the term "hybrid" may be shown on the label. If the percentage of hybrid seed is less than 95, but greater than 75, then the statement "contains from 75 to 95 percent hybrid seed" must be shown on the label. The term "hybrid" may not be used if the pure seed contains less than 75 percent hydrid seed.

Corn and Sorghum Hybrids

THE development of commercial hybrids in most crops, including alfalfa, has followed patterns similar to the ones that evolved with the development of hybrid corn and hybrid sorghum. In reality the history of developing hybrid varieties in any crop is primarily a history of developing pollen controls. Pollen-control characteristics can take many forms such as genetic (nuclear) male sterility, cytoplasmic-male sterility, female sterility, self-incompatibility, dioecious flower production, and mechanical emasculation. Any form of pollen control that can be relied on to produce at least 75 percent intercrossed seed of two parental lines could be utilized in the production of hybrid seed.

The first corn hybrids were developed by utilizing corn's monoecious flower production trait. Two parental lines were planted in alternating strips across a field. The immature tassels were pulled out of the female (seed parent rows) so that all pollen in the field had to come from the non-emasculated parent. All seed harvested from the female rows was hybrid seed. These procedures were effective and economical because of the low labor costs of detasseling in the 1930's and 1940's.

Rhoades (*14*, *15*) described a type of pollen sterility in corn that was inherited through a female cytoplasm. Progeny from a male-sterile X male-fertile cross were primarily male-sterile. The first attempts to utilize this cytoplasmic-male sterile in commercial hybrid production were described by Duvick (*7*). Cytoplasmic-male-sterile hybrids were developed so that single crosses could be produced without detasseling. However, before the procedure was fully utilized, it was observed that several of the supposedly male-sterile lines shed pollen under some environments. This indicated to the breeders that cytoplasmic-male sterility was unstable. The enthusiasm for utilizing Rhoade's male-sterile line faded and consequenty it was lost.

A new, more reliable sterile type of cytoplasm was described by Jones and Everett (*8*). This cytoplasmic sterile was isolated by Rogers in 1944 at the Texas Agricultural Experiment Station and became known as Texas or T. Proof of the interaction between genotype and cytoplasm in corn with respect to pollen sterility was presented by Josephson and Jenkins (*9*) and Jones and Everett (*8*). It was possible for corn breeders to select reliable sterile genotypes from the Texas source of sterility. Furthermore, they found that they could use restorer genes to restore pollen fertility. Combinations of cytoplasmic sterile cytoplasm and genetic-restorer genes allowed the production of doublecross corn hybrids with no detasseling. This system was so successful that prior to the corn blight epiphytotic in 1970 nearly all corn hybrids were produced by this system.

The first scheme for producing sorghum hybrids was suggested by Stephens (*17*) when he reported the discovery of a gene for male sterility. In that scheme, one-half of the female flowering heads would be fertile and would have to be removed before pollination. The cost of this rogueing operation was so great that it prohibited its use. When Stephens et al. (*18*) found a second male-sterile gene, a scheme was devised for producing a 3-way cross. Commercial 3-way crosses were just getting into production when Stephens and Holland (*19*) discovered that cytoplasmic-male sterility resulted from

the introduction of Kafir chromosomes into Milo cytoplasm. Hybrid sorghum seed production developed rapidly after this discovery.

This brief outline of the development of corn and sorghum hybrids emphasizes some of the difficulties involved with pollen control and the importance of cytoplasmic-male sterility to the successful commercial production of hybrids. In both crops the first attempts at producing hybrids utilized either mechanical or imperfect genetic systems for pollen control. Low-cost production of hybrid seed was not possible until a reliable source of cytoplasmic-male sterility was found. Recently, however, cytoplasmic-male sterility in hybrid corn production has been partially replaced by mechanical detasseling to avoid the disease problem associated with the Texas source of cytoplasm. Successful hybrid seed programs based on cytoplastic-male sterility are now underway in onions, sugar beets, pearl millet, sorghum, and corn. Programs are presently underway to find usable male-sterility systems in many other field crops.

Barriers in Alfalfa Hybrids

THE idea of alfalfa hybrids is not of recent origin. Tysdal, Kiesselbach, and Westover (*20*) in 1942 suggested that the yield of synthetic varieties had an apparent "ceiling" and that this ceiling was below the yield obtained from F_1 hybrids. They also suggested a procedure for commercially utilizing hybrid vigor. It consisted of vegetatively propagating two self-sterile alfalfa plants on about 25 acres and allowing the clones to intercross. The F_1 seed from two such sets of crosses would be mixed, planted, and allowed to interpollinate to produce double-cross seed. Partially because of the expense of vegetative propagation and partially because of the lack of plants with dependable self-sterility the scheme was not utilized until 1970 when Farmers Forage Research Cooperative produced the variety Tempo. Tempo has been called an uncontrolled double-cross by its developer. The 1968 legal definition of a hybrid precludes the use of this term for seed of Tempo in commercial channels, because the extent of crossing has not been determined. Myers and Rudorf (*11*) suggested that if cytoplasmic male steriles could be found it should be possible to produce alfalfa hybrids. Based on the historical development of corn and sorghum hybrids, it appeared that the discovery and utilization of cytoplasmic-male sterility held the key to production of alfalfa hybrids. However, the development of hybrid alfalfas has not been that simple. Alfalfa differs from corn and sorghum in its method of pollen dispersal, complexity of inheritance patterns, and nature of growth habit. The first two of these three factors have added barriers to the rapid development of hybrid alfalfa that did not exist with either corn or sorghum.

Corn and sorghum pollen is wind disseminated, whereas alfalfa pollen must be transported by insect pollinators. In the alfalfa flower the staminal column (stamens and pistil) is held inside the two keel petals under tension. Bees foraging for nectar or pollen apply pressure to the flower so that the staminal column is released (tripped). When the stamens and pistil trip forward, they usually strike the pollinating bee. Pollen adheres to the hairy body of the bee and some pollen from the bee inadvertently rubs off onto the sticky alfalfa stigma. The introduction of foreign pollen onto the stigma is necessary for hybrid seed to be produced. Alfalfa pollination is only incidental to bee activities because the bees are searching for either nectar or pollen. Flower color, aroma, and nectar qualities appear to be important factors in bee preference and can influence pollination patterns. Several species of bees (honeybees, bumblebees, leafcutter bees, and alkali bees) pollinate alfalfa and each species may have a somewhat different set of preference standards.

Differences in pollinator preference among plants within most synthetic varieties has not been considered important in seed production because the varieties have a broad genetic base and the preferred and non-preferred plants usually occur at random in the seed field. A broad genetic base in a synthetic variety could reduce the potential undesirable effects (i.e., gene shift or low seed production) that could result from differential attractiveness. Conversely, in hybrid production, attractiveness differences can be very important to the economics of producing hybrid seed. In hybrids it is necessary to obtain frequent pollination between rows of male-sterile plants and rows of male-fertile pollenizer plants. If either the male-sterile or male-fertile parent differs greatly in attractiveness, then the bees will concentrate on the most attractive parent and only seldom visit the nonattractive parent. This results in very low hybrid seed yields on the male-sterile rows.

Most pollen-control procedures involve some type of nuclear gene control. Therefore, ease with which genes can be manipulated is important in the development of hybrid varieties. Inheritance patterns in alfalfa are much more complex than those in either corn or sorghum. Corn and sorghum are both diploid species and thereby contain two set of homologous chromosomes, whereas alfalfa is a tetraploid species and contains four sets of homologous chromosomes. In diploid species, each gene may be found in any one of three genotypes, AA, Aa, and aa. In a tetraploid species, each gene may be found in any one of five genotypes, AAAA, AAAa, AAaa, Aaaa, and aaaa. The increase in chromosome sets and subsequent increase in genotype number that is present in tertaploid species greatly increases the number of plants that a plant breeder must grow in order to select plants with specific genotypes. The proportion of homozygous recessive plants expected from crosses between diploid genotypes are 1 in 4 for Aa x Aa, 1 in 16 for Aa Bb x Aa Bb, and 1 in 64 for Aa Bb Cc x Aa Bb Cc. In contrast, the proportion of homozygous recessive plants expected from crosses between tetraploid genotypes are 1 in 36 for AAaa x AAaa , 1 in 1,296 for AAaa BBbb x AAaa BBbb, and 1 in 46,656 for AAaa BBbb CCcc x AAaa BBbb CCcc.

Not all characteristics of alfalfa are barriers to hybrid seed production. The perennial growth pattern and utilization of the crop as a forage are advantages for alfalfa. Corn and sorghum are both grain crops with annual growth patterns. Their pollen-sterility systems must therefore be maintained as seed lines and the hybrid seed must contain fertility-restorer genes so that growers can depend on a seed crop. In contrast, superior alfalfa parents can be vegetatively propagated and, since the hybrid will be used as forage, no concern needs to be given to fertility of the hybrid plants. The perennial growth habit of alfalfa also allows seed fields to remain in production for several years without reestablishment thereby amortizing the original establishment cost over several years.

Alfalfa Pollen Controls

THE evidence for a self-incompatibility system in alfalfa was summarized by Barnes et al. (*2*). They concluded that some type of self-incompatibility system was present in alfalfa but it differed from the prototype systems described for *Trifolium, Nicotiana, Oenothera, Petunia,* and quayule (*Parthenium*) in two major respects. First, the self-incompatibility reaction in alfalfa only partially prevents self-fertilization. Second, there may be some inhibition of selfed pollen-tube growth within the style, however, the principal manifestation of self-incompatibility is the pollen tube-ovule interaction within the ovarian cavity. Regardless of the mechanism involved, self-incompatibility in alfalfa is not considered a reliable method of pollen control for hybrid production. If it were used in a scheme such as the one proposed by Tysdal et al. (*20*), gene markers would be needed to identify the degree of selfing and sibbing. A new development that might be useful in the production of hybrids using self-incompatibility is the finding that selfed seed is about 5 percent smaller than sibbed seed and sibbed seed is about 5 percent smaller than outcrossed (hybrid) seed (*12*). It should, therefore, be possible to remove the smaller seeds from a lot with less than 75 percent hybrid seed and upgrade that lot so it could meet the minimum percentage of hybrid seed count. Gene markers would be necessary for this procedure to be effective.

The first reliable pollen-control mechanism reported in alfalfa was a form of male sterility characterized by an atypical behavior immediately after the microspores were released from the tetrads (*4*). The tapetum and microspores degenerated leaving only shrunken and empty anthers. McLennan and Childers (*10*) found that this 20 DRC type of sterility was controlled by one gene (ms_3) with tetrasomic inheritance. The parent clone (20 DRC) and its male-sterile progenies have been used in many studies throughout North America as a seed parent in the production of experimental hybrids. Unfortunately this clone was not well adapted to most alfalfa growing areas and lacked many types of disease and insect resistance required for superior performance. Most experimental hybrids using the 20 DRC type of sterility were inferior to present varieties. This, plus the fact that schemes for producing hybrids using a genetic male sterile required large acreages of vegetative propagation, caused most plant breeders to dismiss the production of hybrids with this type of pollen control as being impractical.

The first documented evidence of cytoplasmic-male sterility in alfalfa was by Davis and Greenblatt

479-943 O - 72 - 3

(*6*) in 1967. This was followed by reports from Bradner and Childers (*3*) and Pedersen and Stucker (*13*). The cytoplasmic-male-sterility system was characterized by incomplete pollen development or abortion of pollen grains after completion of meiosis. Unlike the complete male sterility of the 20 DRC genetic system the cytoplasmic system was characterized by various degrees of partial sterility. These ranged from only a few normal grains of pollen to nearly normal amounts of pollen per anther. According to Barnes et al. (*2*), male-sterile plants described by both Davis and Greenblatt (*6*) and Pedersen and Stucker (*13*) were controlled by the same cytoplasmic system. Barnes et al. suggested that complete cytoplasmic-male sterility in alfalfa was controlled by one homozygous recessive nuclear gene interacting with sterile cytoplasm. Incomplete or partial sterility was reported to be controlled by the nuclear sterility gene being in the simplex (Aaaa), duplex (AAaa), or possibly triplex (AAAa) genotypes. Duplex genotypes would have more normal pollen than simplex genotypes when in combination with sterile cytoplasm. Bradner and Childers (*3*) also proposed that one gene in combination with sterile cytoplasm was the probable basis for sterility.

The first commercial alfalfa hybrids utilizing cytoplasmic-male sterility were marketed in the Midwest by the L. Teweles Seed Co., Milwaukee, Wis., in 1968.[1] On March 16, 1971, a U.S. Patent, No. 3,570,181, was issued to the L. Teweles Seed Co. on the hybrid-production process. Presumably, this patent precludes the use of cytoplasmic-male sterility for all commercial production of future alfalfa hybrid varieties in the United States without payment of royalties.

The Future for Hybrids

A USABLE pollen-control system is available in alfalfa and commercial hybrids that are being produced. However, the potential of alfalfa hybrids has not been determined. Problems remain to be solved before the final verdict can be given.

Many types of crossing plans could be utilized in hybrid production. To date the most workable plan has been the production of three-way hybrids according to the following scheme:

$$A(\text{ms}) \times B \rightarrow AB(\text{ms}) \times C \rightarrow ABC \text{ commercial hybrid}$$

In this scheme:

- *A* is a male-sterile clone or seed propagated line;
- *B* is a male-fertile, sterility maintainer clone or seed propagated line; and
- *C* is a male-fertile pollen parent (pollenizer) that can be either a sterility maintainer or fertility restorer line. The term "pollenizer" rather than "pollinator" was suggested for describing male fertile parents so as not to confuse the source of pollen with the pollinating insect.

[1] Trade names are used in this publication solely for the purpose of providing specific information. Mention of trade names does not constitute guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

The commercial hybrid production practice has been to plant four rows of male steriles spaced 40 inches apart, then leave a blank row, plant four rows of pollenizer, then leave a blank row, plant four rows of male steriles and etc. Seed produced by this system is several times more expensive than seed of synthetic varieties. One reason for the increased cost is that seed yields on the male steriles have usually been less than 50 percent of the male pollenizer. This is due in part to spatial isolation of the male-sterile and male-fertile rows. Usually the two male-sterile rows adjacent to pollenizer rows have more seed than the two non-adjacent rows. Differences in attractiveness between the male-sterile and pollenizer rows could also reduce hybrid seed set. The fact that hybrid seed is produced on less than half of a field also adds to the expense.

Even though a usable pollen-control procedure has been developed, it is still a difficult job to find parents that are A-, B-, and C-type plants, that possess all of the needed types of insect and disease resistance, that are attractive to pollinators and produce high seed yields, and that are well adapted and produce increased forage yields. It has been difficult to find good parents for synthetic varieties; therefore, the odds of finding good parents with A and B characteristics in addition are much more remote. In alfalfa the B-type parents are the most difficult to find and identify because they occur infrequently and can only be identified by crossing them onto an A-type plant and observing pollen production in the progeny. Until recently it appeared that many of the disease and insect resistant traits could be incorporated into a hybrid by using a resistant C parent. This was because those traits were inherited in a dominant manner. However, Barnes et al. (*1*) reported that resistance to Phytophthora root rot, a serious disease in many areas, was conditioned by a recessive gene. This means that all three parents in a hybrid must be resistant, not just the C parent.

Increased seed cost is another hurdle that must be considered with hybrid alfalfa. According to W. H. Davis of L. Teweles Seed Co. (*5*), 1970 retail seed prices of $1.66 to $2.08 per pound would be charged for hybrid seed as compared to $0.50 to $0.86 for present synthetic varieties. He estimated that a 10 percent yield gain of the hybrids would justify the $1.66 price of seed and a 20 percent yield gain could justify the $2.08 price. Alfalfa seed is usually planted at a rate of 12 to 15 pounds per acre in the upper Midwest. Seeding rates increase as one goes south.

During the last 3 years the forage yields of available hybrids have been compared with the forage yields of available synthetic varieties in most of the 12 North Central States. The performance of some hybrids has been good in some tests. However, in general, they have not performed better than the new synthetic varieties. Contrary to some of the early speculation, it is now apparent that hybrid alfalfa varieties will not be superior just because they are hybrid. They must first have multiple pest resistance and must be well adapted to an area before the potential advantage of hybridity can be recognized.

The advent of hybrid alfalfas has created other types of challenges and problems. For example, precedents were not available to establish seed certification standards for either minimum isolation distances or for the amount of pollen leakage allowable in the male-sterile portion of the seed-production field. These types of problems are complex and require a great deal of long-term cooperative research among Federal, State, and industry scientists for solutions. Prior to the issuance of the hybrid production process patent, there were many agencies and scientists willing to help develop methodology for developing better hybrids. However, many of them may be hesitant to continue research in this area because it may benefit some segments of the alfalfa industry more than others.

The evolution of hybrid alfalfa, like life itself, is still in the process of change. Eventful chapters of the story remain to be written in the future.

LITERATURE CITED

(*1*) Barnes, D. K., Frosheiser, F. I., and Shyh-Jane Nancy Lu.
1971. Breeding alfalfa for phytophthora root rot resistance. Agron. Abstracts p. 3.

(*2*) Barnes, D. K., Bingham, E. T., Axtell, J. D., and Davis, W. H.
1972. The flower, sterility mechanisms, and pollination control. *In* C. H. Hanson (ed.) Alfalfa Science and Tech-

nology. Agron. 15:123–141. Amer. Soc. of Agron., Madison, Wis.

(3) Bradner, N. R., and Childers, W. R.
1968. cytoplasmic male sterility in alfalfa. Can. Jour. Plant Sci., 48:111–112.

(4) Childers, W. R., and McLennan, H. A.
1960. inheritance studies of a completely male sterile character in medicago sativa l. Can. Jour. Genet. Cytol., 2:57–65.

(5) Davis, W. H.
1970. what is the future of hybrid alfalfa? Proc. Third Research-Industry Conference, American Forage and Grassland Coun., State Col., PA. 77 p.

(6) Davis, W. H., and Greenblatt, Irwin M.
1967. cytoplasmic male sterility in alfalfa. Jour. Hered., 58:301–305.

(7) Duvick, D. N.
1959. the use of cytoplasmic male sterility in hybrid seed production. Econ. Bot., 13:167–195.

(8) Jones, D. F., and Everett, H. L.
1949. hybrid seed corn. Connecticut Agr. Exp. Sta. Bull. 532.

(9) Josephson, L. M., and Jenkins, M. T.
1948. male sterility in corn hybrids. Jour. Amer. Soc. Agron. 40:267–274.

(10) McLennan, H. A., and Childers, W. R.
1964. transfer of genetic male sterility from tetraploid to diploid alfalfa and inheritance at the diploid level. Proc. Can. Soc. Agron., p. 79.

(11) Myers, W. M., and Rudorf, Wilhelm.
1959. kleeartige futterpflanzen. Hanbuch der Pflanzenzuchtung, 2, Aufl. Band 4, p. 103–213.

(12) Pedersen, M. W., and Barnes, D. K.
1972. alfalfa seed size an indicator of hybridity. Crop Sci. (In press).

(13) Pedersen, M. W., and Stucker, R. E.
1969. evidence of cytoplasmic male sterility in alfalfa. Crop Sci., 9:767–770.

(14) Rhoades, M. M.
1931. cytoplasmic inheritance of male sterility in zea mays. Sci., 73:340–341.

(15) ———
1933. the cytoplasmic inheritance of male sterility in zea mays. Jour. Genet., 27:71–95.

(16) Stahmann, Mark A.
1968. the potential for alfalfa protein concentrates in animal and human nutrition. Proc. Fourteenth Annual Farm Seed Conference, Amer. Seed Trade Assoc., Wash., D.C., 67 p.

(17) Stephens, J. C.
1937. male sterility in sorghum: its possible utilization in production of hybrid seed. Jour. Amer. Soc. Agron., 29:690–696.

(18) Stephens, J. C., Kuykendall, Glen H., and George, Donald W.
1952. experimental production of hybrid sorghum seed with a three-way cross. Agron. Jour., 44:369–373.

(19) Stephens, J. C., and Holland, A. F.
1954. cytoplasmic male sterility for hybrid sorghum seed production. Agron. Jour. 46:20–23.

(20) Tysdal, H. M., Kiesselbach, T. A., and Westover, H. L.
1942. alfalfa breeding. Nebraska Agrl. Exp. Sta. Res. Bull., 124, 46 p.

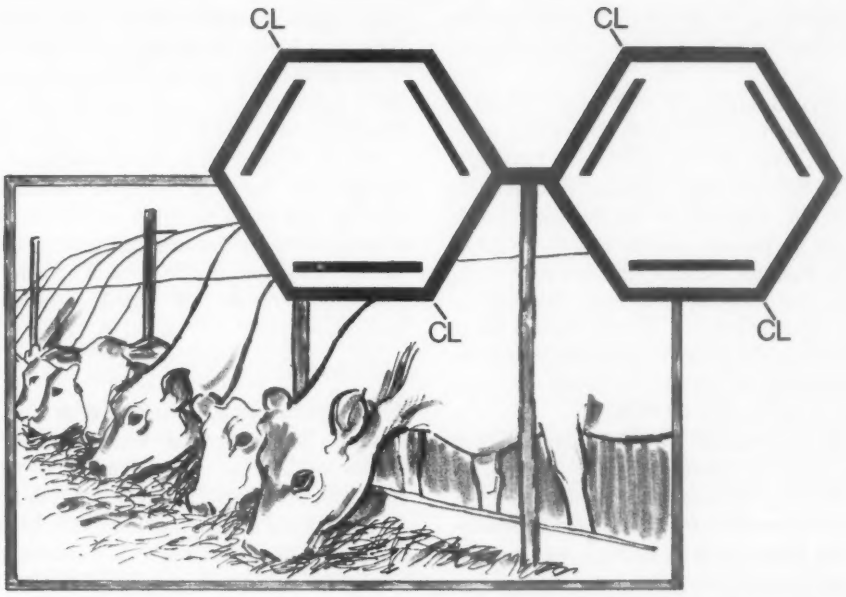
TEXAS A. & M. ESTABLISHES NEW RESEARCH CENTER

TEXAS A. & M. University acquired the land, buildings, and staff of Texas Research Foundation at Renner, Tex., on September 1. The new 380-acre research and extension center is located near Dallas.

Referring to the new center, Dr. H. O. Kunkel, dean of the Texas A. & M. College of Agriculture and director of the Texas Agricultural Experiment Station, said: "We are depending on the Foundation staff—assembled over the years by Dr. C. L. Lundell—to continue its excellent endeavors without interruption."

Scientific research in progress on various crops, forages, and land management systems will continue at the site, Kunkel noted, pointing out that as staff is added, agricultural research will be expanded to meet needs of a changing agriculture in the region.

S. H. Whitehurst, administrator of Texas Research Foundation, serves as superintendent of programs of the Texas Agricultural Experiment Station at Dallas and coordinator of the agricultural programs of Texas Research Foundation transferred to Texas A. & M.



PCB RESIDUES: THEIR SIGNIFICANCE TO ANIMAL AGRICULTURE

G. F. FRIES

POLYCHLORINATED biphenyls, more commonly known as PCB's, are chlorinated hydrocarbon compounds that have a variety of industrial uses. Residues of these compounds have been found to be widely distributed in the environment.

These residues have occurred in a number of foods but more frequently in seafoods than any other food product. Contamination of animal products has not been widespread, but several significant localized problems have arisen with poultry and dairy products.

It is my purpose to review the known incidents of poultry and milk contamination by PCB's and to discuss future problems that may arise.

Background

PCB's have been produced since 1930 but were not recognized as widespread environmental contaminants until 1966. The major use of PCB's has been as a dielectric in transformers and capacitors. Other significant uses have included heat transfer and hydraulic fluids. Minor uses, making up less than 6 percent of sales, have included such items as surface coatings, plasticizers, sealants, adhesives, and printing inks.

The sole producer of PCB's in the United States is the Monsanto Chemical Co.[1] whose production of these compounds peaked in 1970 at about 36,500 tons. The company has restricted sales of PCB's to use in sealed products such as a dielectric in transformers and capacitors. Foreign producers of PCB's

[1] Trade names are used in this publication solely for the purpose of providing specific information. Mention of trade names does not constitute guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

are not a significant factor in U.S. usage but are important when considering global contamination problems.

Polychlorinated biphenyls and polychlorinated triphenyls are sold by Monsanto under the trade name Aroclor. They are described by a four digit number. The first two digits indicate the chemical structure of the material, while the second two digits indicate the percent chlorine that the material contains. For example, Aroclor 1254 is a polychlorinated biphenyl containing 54 percent chlorine.

Aroclors are mixtures but do not contain all of the 210 theoretically possibly polychlorinated biphenyl compounds. A single formulation may contain as many as 40 individual PCB's.

Chemically and biologically, PCB's are similar to other chlorinated hydrocarbon compounds. PCB's can be isolated by the same analytical procedures used in routine surveying for chlorinated hydrocarbon pesticides. Some components have the same gas chromotographic retention times as chlorinated hydrocarbon pesticides. These interferences led to the discovery and identification of PCB's in the environment. Some values listed in the literature as DDT prior to 1966 may have been PCB's.

PCB's persist in the environment and resist degradation by organisms. However, in some species the less chlorinated components are degraded and converted to water soluble compounds. PCB's are stored in the body fat of animals where the concentrations undergo biological magnification. Thus, animals at the higher levels of the food chain will have the highest residue levels.

The toxicological significance of PCB residues has not been determined. The only clinical experience with acute human exposure occurred in Japan in 1968. About 1,000 persons had eaten rice oil contaminated with PCB's. They developed darkened skins, eye discharge, severe acne, and symptoms of what came to be called Yusho disease. Average intake was 2 grams of PCB, with symptoms appearing at a minimum dose of 0.5 g.

A variety of subacute effects at much lower intakes have been observed in many other species. The long-term significance of these effects and their implications for human health has not been determined.

Toxicological evaluation of PCB's is difficult because they all are mixtures. Proportions of the components are different among the various Aroclors. One cannot evaluate a single formulation and assume that the result applies to other formulations. There is also evidence that PCB's, particularly those of European and Japanese manufacture, may contain highly toxic impurities such as dibenzofurans.

The PCB problems in the United States involving animal agriculture have been of two distinct types. The poultry incidents were short-term, high-level exposures causing not only high residues in the product but also adverse effects on the birds. The milk incidents were chronic exposures involving only residues.

Milk Residues

PCB residues in milk were first detected in 1970 in Ohio. The source was traced to a sealant used on the inside walls in the manufacture of concrete stave silos. Aroclor 1254 was included in the formula of this sealant which was used extensively by one company. All incidents of milk contamination have involved the silos of this company and its successors.

Other companies have in their files formulas for PCB-containing sealants. It has not been determined whether or not any of these were produced and used. However, no contaminated silos originating from other companies have been detected.

The sealant containing PCB's was developed in the 1930's and used until about 1968. Estimates of the number of silos in which it was used are not available. In addition to Ohio, contaminated silos exist in Kentucky, Tennessee, and adjacent States.

Scrapings from silo walls contain as much as 1 to 2 percent PCB. While PCB's are not very soluble in water, their concentrations as high as 100 p.p.m. in the silage dry matter can occur adjacent to the wall. Levels drop rapidly as the distance from the wall increases and significant residues are seldom found more than 2 or 3 feet from the wall. The diffusion towards the center is greater at lower levels in the silo.

Despite the relatively low transfer of PCB from the wall to the silage, there are two factors in addition to the high-wall concentrations that contribute to the milk residue problem. First, it is impractical to separate the high-residue silage adjacent to the wall from the other silage and second, PCB's are transferred very efficiently from the silage to the cows' milk.

We surveyed a number of farms that were using contaminated silos. Levels of PCB residues in milk

fat were approximately 10 p.p.m. when the silage was fed. Other workers have found individual farms with residue levels as high as 28 p.p.m. The Food and Drug Administration (FDA) guideline for maximum PCB residue is equivalent to 2.5 p.p.m. in milk fat. Low-level silage feeding would theoretically allow production of milk within the guidelines, but the level is too low to be practical.

We have studied the metabolism of PCB's by cows both in the field and under controlled conditions in the laboratory. Transfer of PCB's from the feed to the milk fat and body fat is very efficient. Rates of transfer are approximately equal to the rates for DDE, the most persistent metabolite of DDT. The total diet of the cow must contain less than 0.5 p.p.m. PCB in order to prevent milk from exceeding FDA guidelines.

If excessive residues occur in milk the time required to return the herd to a marketable condition may be quite long. PCB's are removed from the body fat at a rate proportional to the concentration in the body fat. It takes approximately 6 to 8 weeks to reduce the concentration in milk fat by one-half. Thus, if the PCB concentration in milk fat is 10 p.p.m. (four times the FDA guideline), it will take approximately 16 weeks from the time the contaminated feed is removed from the cow's diet for the milk to be within the guideline.

Since the PCB concentration in the body fat is closely related to that in the milk fat, cows exceeding the guideline for milk would also exceed the guideline for meat. If the cows are producing milk with excessive PCB levels, one can either destroy the animal or wait until the residue level in milk returns within the guideline. In either case the loss to the dairyman can be substantial.

The number of contaminated silos detected by regulatory activity has been small compared to the estimated number of contaminated silos. This is largely due to problems of analysis and survey techniques that are followed by regulatory agencies. Generally the lower level of detection has been about 1 p.p.m. in milk fat. Thus, if the milk supply is surveyed using composite samples of many farms, it would be difficult to detect an individual farm with contaminated milk if the other farms in the sample produced clean milk.

The amount of PCB-contaminated milk produced is relatively small compared to the total milk supply of the country. The farms producing contaminated milk are scattered and it is greatly diluted by clean milk. Thus, there is no reason to be particularly concerned about the effects of the residues on the health of the general public.

However, there is one segment of the population that could receive significant exposures from this source: Farm families who drink milk from their own cows fed on contaminated silage. Using typical residue and milk consumption levels, intakes of PCB for over 15 to 20 years would approximate the level of short-term intakes that caused serious effects in Japan.

However, the problem is probably not as serious as implied since a dose consumed over a long period may be less serious than the same dose consumed in a very short period. Also the PCB in the contaminated silos was more chlorinated than the Japanese product which also may have contained highly toxic contaminants. Thus, the situations are not directly comparable.

Silo Decontamination

ANY method devised to correct the PCB problem in milk should have two desirable characteristics: Effectiveness and economy. Because of the high efficiency of transfer of PCB's from a cow's diet to its milk there is no known effective remedy short of elimination of most PCB from the cow's diet. The most effective and most costly method of eliminating the problem would be complete abandonment of the contaminated silos.

Several alternatives to complete abandonment have been suggested and partially tested. The suggestions generally involve resealing the silo to block the diffusion of PCB's from the silage. In some cases the resealing may be preceded by partial removal of the PCB's.

Partial removal of PCB has been attempted by either sandblasting the surface or washing the surface with a suitable solvent. Sandblasting followed by resealing has been tried but no studies of its effectiveness have been made. The cost of this procedure is greater than half the cost of a new silo and may exceed the depreciated value of the silo.

Sandblasting has consequences that have not been fully explored. The operation is dusty and thus it is possible that other areas of the farm could be contaminated. The PCB's also penetrate quite deeply into concrete. It is not known how much of the surface would have to be removed to completely

remove or reduce the PCB's to a satisfactory level.

Solvent washing has been tried on a few silos, but the cost of the procedure is not established. Presumably it would be similar to the cost of sandblasting. In field experiments we have found that the process of solvent washing and resealing has not been effective. Whether this was due to the specific procedure used has not been determined.

Resealing the silo without prior treatment would be a desirable method because of its lower cost. For this method to be effective the PCB should not penetrate the new sealant and the new sealant must adhere to the walls for the remainder of the silo's life. Several materials have been tested and some show promise. However, the work that has been reported has only covered the first years' experience. It is not known how well these materials will continue to seal.

Temporary expedients have been attempted on a few farms where contaminated silos were identified by regulatory agencies. Lining the interior of the silo with plastic was effective enough to keep the residue levels near the guideline. Discarding the silage within about 6 inches of the wall was less effective. Neither method is compatible with mechanized unloading of the silo and neither eliminates the problem.

Poultry

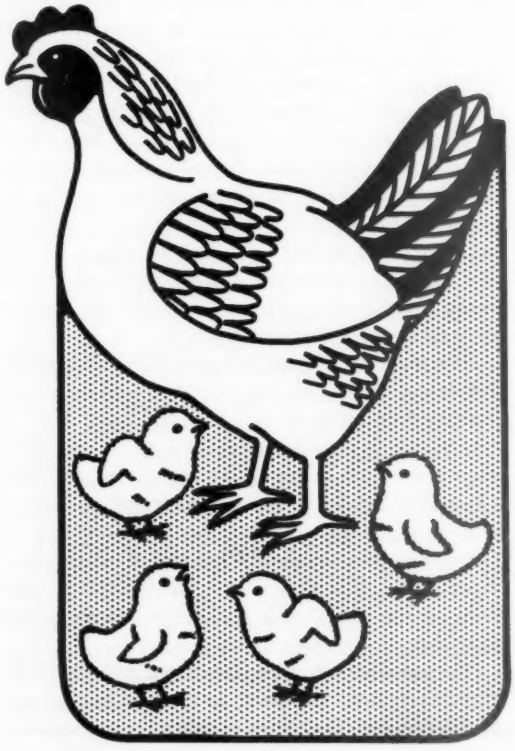
SOME of the most spectacular and publicized incidents of PCB contamination have involved poultry. In the past 2 years there have been serious incidents in North Carolina, New York, Maine, and Minnesota. The source of contamination was not established in all cases.

The North Carolina incident was the most extensive and is the best documented. A large poultry producer noted a severe decrease in hatchability of eggs. The problem was tracked to fishmeal contaminated by Aroclor 1242. The fishmeal was contaminated during processing when the PCB leaked from a corroded heat exchanger. In addition to this incident, contaminated feed from the same source had been shipped to 11 other States before the situation was brought under control. Significant numbers of broilers and eggs had to be destroyed because of the high residues.

Lowered hatchability is one of the more important toxicological effects of PCB's in birds. In contrast to some of the chlorinated hydrocarbons such as DDT, PCB's apparently have little or no effect on eggshell thickness. The effect on hatchability may occur at levels as low as 10 p.p.m. in the diet. Generally, there is full development of the embryo but the chicks are unable to break the shell. Subdermal edema is the most frequent abnormality.

Only the Aroclors with intermediate degrees of chlorination affect hatchability. We found that Aroclor 1232 has some effect, while the most severe effects are encountered with Aroclors 1242 and 1248. In contrast, Aroclors 1221 and 1254 have no effect at levels as high as 20 p.p.m. in the diet. No significant effects of PCB's on mammalian embryo development have been reported. This may indicate a species difference. However, most mammalian studies have used the more highly chlorinated PCB's and it is possible such effects could be obtained with the less chlorinated compounds.

The FDA has recently prohibited use of PCB's in feed plants and the use of recycled paper for feed



containers. Presumably these restrictions should eliminate short-term exposures of the type that have occurred with poultry. However, since PCB's have been so widely used and all of their possible uses have not been established, it is conceivable that a few incidents of this type will occur in the future.

Other Animal Products

PCB contamination of poultry and dairy products suggests that contamination of other animal products may have occurred. However, there are circumstances that minimize its significance and chances for detection.

It is possible that some cattle raised for beef have received silage from contaminated silos. However, silage, particularly during fattening, is not as important a component of beef rations as it is of dairy rations. From levels in milk contamination, it can be assumed that some carcasses of surplus dairy animals have residue levels exceeding the FDA guideline. However, surplus dairy animals account for a small part of the meat supply. There would only be a small chance for a carcass of either type to be selected for routine analysis.

The incidents involving poultry suggest that contamination of feeds for other livestock could also have occurred. Some of the fishmeal from the North Carolina incident may have been used for pigs, yet residues in pork have not been reported. Some of the fishmeal also was ultimately destined for catfish food.

To date there have been no severe losses of species other than poultry due to PCB's. This could indicate that other species are not as sensitive to PCB's as poultry and if exposure occurred, there was no noticeable effect on productivity.

Discussion

PCB residues have been accumulating for 40 years but were not detected until 6 years ago. It can be assumed that other undetected contamination exists. It usually comes to light by one of two ways. One is the release of high levels of a material and subsequent observation of severe problems, as illustrated by the poultry incidents. The other, and more frequent, is the development of analytical instruments and methods capable of detecting smaller residues than was previously possible, as illustrated by the silo incidents.

PCB's have the two characteristics of a compound most likely to cause contamination problems. These are resistance to degradation and solubility in fat. Water soluble compounds, even if not degradable, would tend to disperse in the environment and would be of less potential hazard.

The poultry feed and silo problems also illustrate some practical problems associated with regulatory activities. With the poultry feed there was no question of the severity of the problem. Through an intensive short-term effort, regulatory agencies located all of the contaminated feed and products which were then removed from food marketing channels.

The situation involving silos is considerably different. Mixed samples of milk from several farms rarely show significant residues. Thus, only a small percentage of the affected farms have been identified in routine surveys.

In order to completely eliminate the problem it would be necessary to sample individual farms. This would involve a very large sample load and is probably beyond the capability of most regulatory laboratories. This problem could be overcome to a large extent if a preliminary survey was made and the individual samples were limited to the farms having the suspected type of silo. To my knowledge only Ohio is following such a program at the present time.

Regulatory efforts that are not completely effective raise a number of questions. The first is whether or not the undetected farms pose a threat to public health. They probably do not because dilution would reduce the residues to a negligible level. However, the "dilution principle" has not been acceptable to many regulatory agencies.

The second question is whether or not the occasional removal of a contaminated dairy from the market provides a sufficient incentive for other dairymen to determine whether they have silos that are contaminated and to correct the problem if it exists. Removal from the market is a major economic loss but the cost of correcting the problem without market loss can also be substantial. If a dairyman feels the chances of being detected are very small he may take the chance and ignore the possibility that he has contamination.

Problems are also posed for others, such as research and extension personnel, who may be aware

of the nature of the situation but who have no direct responsibility for its resolution. They should not condone the production of milk with residue levels above the guidelines. On the other hand, it is difficult to recommend a large economic outlay to a dairyman unless it prevents a larger outlay in the future.

Also confusing the issue is the determination of responsibility for the contaminated silos. Litigation involving dairymen, the silo builder, and the PCB manufacturer is now in progress. When responsibility is established, it should be easier to decide upon and to implement a course of action.

ON ANSWERING "WHAT ARE YOU DOING, MR. RESEARCHER?"

I sometimes imagine myself discussing my own (research) project with several kinds of people and they each ask the question, "What are you doing."

Let us say that this question is asked by a fellow scientist, a Texas beef cattle producer, a man in the street, and a member of the legislative Appropriations Committee.

To my fellow scientist I would say, "I have a theory based partially on a feedback control model that the regulation of internal temperature in cattle can be studied by treating the hypothalmus as a sensor-controller amenable to testing. To achieve such tests I have to install a camula permanently in the anterior hypothalmus to permit repeated entry. Thus the tissue can be heated artificially to put a step input into the controller or I can measure its temperature change in response to a step change in environmental temperature. In either case the data for entering the control model can be secured and from this the thermal time constant can be derived. Thus a small advance in our knowledge of bovine temperature regulation might be made."

To the beef cattle producer I would say, "I believe I have worked out a method for rapid identification of heat-tolerant individuals within a breed. These individuals should have better feeding efficiency in our climate, thus making your business more profitable."

To the man in the street I would say, "I am working on a scheme which, if successful, will give the consumer better beef at lower cost."

To the legislator I would say, "Texas cattle feeders can use this information to their economic advantage and thus contribute further to our industrial economy."

Now I know all of this sounds kind of awful. . . . trying to be everything to everybody, but every word of what I said, I think, is true and illustrates the idea that if the researcher cannot discuss these matters with people having all shades of opinion, then maybe he shouldn't be doing that kind of work.

R. F. Stewart,
Distinguished Professor of Engineering,
Texas A & M University

(From a talk at a workshop on research planning and decision making held in Washington, D.C. by the Cooperative State Research Service.)

BREEDING SLASH PINE IN FLORIDA: A SUCCESS STORY

A. E. SQUILLACE, KEITH W. DORMAN, AND RALPH E. McNEES

SINCE the early 1600's when pitch was used to caulk the seams and tar the ropes of wooden ships, the naval stores industry, which produces turpentine and rosin from the gum of living pine trees, has alternated between periods of booming prosperity and near-disastrous declines. Now, research begun 30 years ago at Olustee, Fla., to breed a strain of high-gum-yielding slash pine is helping this presently small but still-valuable industry as well as the pulp, lumber, and veneer industries of the South. Efforts to combine high-gum yields with increased amounts of pulpwood and other wood products have helped provide the basic and applied knowledge that foresters needed to develop the tree improvement program throughout the region.

The naval stores industry is located in the slash pine belt of Southeast and, despite recent improvements in equipment and techniques, still operates according to the principles formulated centuries ago. Gum is extracted from mature trees by wounding them periodically, then collected from containers hung on the trees, hauled to a central point,

and distilled by heating to separate the turpentine from the rosin. After gum is extracted for a few years, the trees are sold for pulpwood, logs, or other products and a new stand is planted. Before the advent of obtaining turpentine and tall oil as by-products during the pulping process, the pine forests in this region of the United States produced 75 percent of the world's supply of naval stores products.

DEVELOPING A HIGH-YIELDING STRAIN

Search for Superior Stock

THE research effort to breed a high-gum-yielding strain of slash pine was initiated in 1941 by three Forest Service scientists at Olustee—Harold L. Mitchell, Keith W. Dorman, and C. S. Schopmeyer. These researchers sought to capitalize on previous findings that many trees growing side by side, although of the same size and with the same crown characteristics, yielded vastly different amounts of gum. They therefore suspected gum yields to be genetically controlled. The first step in the breeding program they initially conceived thus consisted of finding high-yielding trees in the woods for use as source material.

In the spring of 1942, a call went out to foresters, naval stores workers, farmers, and others to mark trees that yielded unusually large amounts of gum. After each such tree was located, the researchers compared its weekly yields with those of 30 to 40 average trees nearby. As trees produce only a few ounces of gum per week, it was necessary to extend each assessment over several weeks. By the end of the summer, the researchers had located a dozen trees that produced 2 to 2½ times as much gum as did average trees.

Attempts at Large-Scale Propagation

SIMULTANEOUSLY, these pioneers in tree breeding attempted to find an easy, relatively cheap large-scale method of propagating slash pine vegetatively (asexually, as in rooting or grafting). They felt that the best way to capitalize on the selected high yielders was to produce vegetative propagules from them for future planting by forest landowners, much as grafting and rooting had been successfully used with fruit and nut trees. Past results with fruit crops indicated that sexual propagation might produce highly variable progenies, with no assurance of high yields. Vegetative propagation, on the other hand, insures no change in the genotype (the individual's hereditary constitution).

Most of the work was done on rooting of cuttings, and this concentrated effort continued for about 5 years. Despite the failure of rooting chemicals to stimulate pine tissue and the difficulty of maintaining turgidity in cuttings whose basal ends become sealed off with pitch exudations, some degree of success was obtained, especially when cuttings were taken from young trees. Much basic information was derived from this. A few rooted cuttings from each of the 12 originally selected high yielders were planted in 1943 to preserve the type. These have been useful in demonstrating that growth characteristics and yielding ability are genetically controlled and that observable differences in these traits among forest trees are not solely attributable to environmental factors as foresters had previously believed, a finding of tremendous importance not only to the breeding program at Olustee but to the tree breeders everywhere. However, even with additional work conducted in the early 1950's, no successful technique was found for the rooting of cuttings on a large scale. More successful was the work in the fifties on methods of grafting, and these methods are now widely used in tree breeding.

Breeding and Progeny Testing

EARLY in 1943, the scientists decided to cross-breed the selected high yielders by using controlled pollination. This procedure would determine to what degree the selections transmitted superiority to their progeny and whether some of the progenies yielded even greater amounts of gum than their parents. By transferring pollen from the male catkins of one tree to the female cones of another, high yielders were mated with other high yielders and with average yielders, and average yielders were mated with one another. Seeds of the resulting families were then sown, and the seedlings produced were outplanted in a replicated test plantation in 1946—probably the first true progeny test of pines ever conducted.

In 1954 and again in 1956, the plantation was "microchipped." This abbreviated chipping technique, which is similar to that used in breeding rubber trees in the Far East, assesses gum-yielding abil-

ity of young trees 4 to 6 inches in diameter. Results were spectacular. The progenies of high-yielding parents produced from 50 to 100 percent more gum than did those of average parents. Some individual trees produced several times as much gum as average trees in wild stands.

Beginning in 1964, the progeny were chipped by commercial procedures for 4 years on one side of the tree and then for 4 additional years on the opposite side. During the 8-year period, the superiority of progeny of high-yielding parents over those of average-yielding parents fluctuated between 77 and 106 percent. These results dispelled the fear that high yielders might "bleed themselves out" of their superiority. Rather, they held up remarkably well.

Seed Orchards

BECAUSE of the difficulty of mass-producing rooted cuttings and the subsequent success with the progeny plantation, the tree breeders turned to the concept of clonal seed orchards. (A *clone* is a group of plants or *ramets* produced by vegetative propagation of a single plant, the *ortet*.) With this technique, grafts from selected trees are planted in an isolated area especially designed to produce large quantities of seed by natural cross-pollination. The resulting seedlings are easily grown in nurseries and transplanted to forest lands.

In 1956–57, a 5-acre demonstration orchard was established from selected trees in the progeny plantation. Each selection possessed high yielding ability and good growth and crown form. This orchard, which is currently producing seed in commercial quantities, is probably the first clonal orchard ever to be established from trees of proven ability. In 1958, a second, larger orchard was established from the progeny plantation in cooperation with the Georgia Forestry Commission, and additional seed orchards totaling 110 acres were established from selections in this plantation during the early 1960's in cooperation with State and private agencies. At full production, the latter orchards are expected to yield about 2,400 pounds of seed annually, sufficient to plant 95,000 acres of high-yielding stock at a spacing of 15 by 15 feet.

Nursery seedlings from seed produced in these orchards are now available for commercial planting in the Southeast. In Georgia, about a million of these seedlings were offered for sale for the 1971–72 planting season; in Florida, about 100,000 were offered; and Louisiana will have them available in the near future. At maturity, these trees will produce 1½ to 2 times as much gum as will average trees, as well as increased amounts of wood, tall oil, and turpentine when harvested for pulpwood. Thus, the high-yielding strain will not only increase the productivity of the naval stores industry but will provide increased returns of other products.

SUPPORTING STUDIES

Intensive Culture

WITH the development of the high-yielding strain well underway, efforts were begun to raise gum yields still higher by applying cultural practices more intensive than those normally used in growing forest trees. In 1958, irrigation, fertilization, and cover cropping were applied to an experimental gum orchard of air layers from high- and average-yielding ortets. (Air layers are vegetative propagules produced by forcing branchlets to form roots while still attached to the tree.) Because of the unusual feature of genetic uniformity within the various types of trees, this experimental orchard has been useful in assessing the effects of cultural practices not only on gum yield but also on such tree characteristics as growth rate, branching habit, and seed production—information which is now being used in breeding other pine species for a wide variety of wood products.

Naval Stores Management

IN the course of the progeny testing discussed earlier, the scientists noted that gum yields of individual trees during the first few months of a naval stores cycle (normally 4 years) were strongly correlated with yields obtained over the entire period. This correlation provided an opportunity to capitalize on variation among individual trees in commercial operations. Because much of the cost involved in chipping is fixed, it would be cheaper (per unit of gum produced) to chip high yielders than low yielders. With a method now available for determining yielding ability early in the naval stores cycle, costs could be reduced by ceasing to chip low yielders as soon as they were identified. Detailed procedures for taking advantage of these findings

were developed and passed on to naval stores operators.

Gum Composition

IN addition to variability in the gum-producing capacity of the tree, the Olustee scientists also explored variability in the composition of the gum itself. In distilling, pine gum is not only broken down into its two components—resin acids (or rosin) and monoterpenes (or turpentine)—but is further refined to valuable organic chemicals such as β-pinene. Thus, it was important to determine whether the gum from high yielders differed in chemical composition from that of ordinary trees. Furthermore, variability in gum composition might hold the key to the control of certain insects that are attracted or repelled by odor. Early testing begun in 1943 indicated that the gum of high yielders produced normal proportions of turpentine and rosin.

More intensive analyses of the turpentine fraction of the gum were conducted in the 1960's in cooperation with Gordon S. Fisher of the Agricultural Research Service's Naval Stores Laboratory in Olustee. Gas chromatography was used to determine the turpentine composition of about 150 trees, including clones and families of known yielding ability. No correlation with gum yield was found, but striking variation was found among individual trees. For example, gum from the cortical tissue of branch tips was discovered to have five major constituents, with the amounts often varying from less than 10 percent to as high as 70 percent. Furthermore, the investigation revealed that the quantities of most constituents are inherited, a finding which may lead to selection and breeding for specific composition.

Of special significance to the tree breeders was the fact that this discovery also provided a tool for identifying relatives in trees. Later work has shown that the contents of at least four of the five major monoterpenes in gum from cortical tissue are simply inherited through single genes. Using these as genemarkers, much as blood types are used to identify relatives in human beings, the scientists can now check the identity of mislabeled ramets and individuals in families.

Strong racial patterns were also detected, a discovery which may allow turpentine composition to be controlled by segregating gum from different geographic areas. Racial patterns in monoterpene composition are so distinct that the seed source of a plan-

Pollinating slash pine in Olustee Experimental Forest.

Bagged flowers are pollinated with pollen from a syringe which punctures the bag; tape is used to reseal bag, then when flower scales have closed and the conelet is no longer receptive to pollen, the bag is removed.

tation can now be identified by sampling the gum from a portion of its trees. This capability will also be useful in verifying the purported origin of seed purchased on the open market.

As noted, most of the early work was concentrated on gum exuding from wounds in the cortical tissue of branch tips. Gum in the stem xylem is, of course, more important commercially, and its composition differs appreciably from that of cortex gum. But when stem gum was sampled, a serious problem was encountered—trees of the same genotype but of different ages were found to differ in gum composition, even though samples were taken at the same height above ground. This difficulty temporarily halted sampling of stem gum. A recent investigation, however, has shown that the composition of stem gum varies with distance from the base of the crown and that comparisons of the composition of stem gum among trees could best be made if samples were taken at the base of the crown. Thus, genetic study of the composition of stem gum has been resumed.

Recently, the scientists have also studied variability in the content of levopimaric acid, one of the resin acids of pine gum that is of commercial importance. In cooperation with Glenn W. Hedrick of the Agricultural Research Service's Laboratory in Olustee, they have detected considerable variation in the content of this acid among trees, with high yielders containing larger amounts of the chemical than low yielders.

Yield of Chemical Extractives

THE Olustee scientists have also recently initiated studies to determine the variation and inheritance of the extractives content of wood and to look for a correlation with gum-yielding ability. Although the sulphate turpentine and tall oil (resins and fatty acids) extracted in some pulping processes are by-products, they are of considerable economic importance to certain manufacturers. As was the case with levopimaric acid, the study showed that high-yielding trees contain more extractives than do low yielders. This unexpected bonus has appreciably increased the value of the high-gum-yielding strain.

"Instant" Progeny Tests

AS slash pines cannot be commercially chipped for gum until they are about 20 years old, it was advisable to devise progeny tests to assess gum-yielding ability at early ages. With the microchipping technique, which involves sowing progeny seed in nursery beds and uprooting the resulting seedlings and transplanting them in the field, the scientists had to wait 8 to 10 years before the trees were large enough to evaluate their gum-yielding abilities. With a recently developed technique called "minichipping," however, this time lag is reduced to 3 years. Under this new technique, progeny seed are sown in peat pellets in a greenhouse. Soon after germination, the pellets are set out on a well-prepared site at a spacing of 20 by 40 inches. The plot is then cultivated, fertilized, and irrigated. Early growth under these conditions is so rapid that the trees attain heights over 10 feet in 3 years and can then be evaluated for yielding ability by collecting gum from tiny wounds in the stem. The reliability of the technique has been established from tests conducted with progeny of known yielding ability, and the technique is now routine for the screening of families.

Racial Variations in Slash Pine

IN any tree improvement program, tree breeders should be aware of the nature and extent of racial variation in the species and also of the existence of interaction between seed source and environment. That is, they should know whether or not trees in different environments and geographic locations vary genetically and whether they will respond differently when planted in contrasting environments with different soils, weather conditions, or insects and diseases.

At Olustee, these questions were thoroughly studied as they related to slash pine. Although no appreciable racial variation was found for gum-yielding ability, distinctive patterns were found for survival, growth, and, as noted previously, turpentine composition. This discovery led to an hypothesis, which may apply to some other species as well: Trees growing in that environment which is most suitable for the species are genetically superior for growth rate, and gradual changes in this trait are apparent as the distance from that environment increases. In formulating the hypothesis, however, the researchers acknowledged the possibility of strong interactions when seeds are moved to greatly different environments. In slash pine, the interactions are relatively minor, an exception being that mortality is greater among seedlings grown from seed moved northward for appreciable distances than among seedlings grown from local seed.

Discovery and Use of Mutants

WORK with inbreeding or selfing (pollination of female cones by male catkins from the same tree) has resulted in the discovery of mutations which are proving useful to tree breeders at Olustee and elsewhere. In a study to determine whether genetic variation in gum yield could be increased by inbreeding, several trees were selfed and the progenies of one of them were found to contain one mutant seedling for every three normal seedlings. These mutants had lethal deficiencies of chlorophyll in the cotyledonary stage. Recognizing the potential usefulness of this mutant as a gene-marker, the investigators later noted that 11 out of about 200 parent trees from which wind-pollinated seeds were collected subsequently produced chlorophyll-deficient seedlings. Because they knew that such seedlings must ordinarily result from selfing, they made the necessary counts and estimated the degree of natural selfing in splash pine to be about 7 percent, the first such estimate ever made for the species. This information provided a basis for determining the degree of inbreeding in seed orchards, where selfing among genetically identical trees can reduce the yield of seed and result in seedlings with lowered growth rates.

The work with gene-markers also stimulated the interest of E. Carlyle Franklin, a graduate student at North Carolina State University who later joined the Olustee staff. In cooperation with the Olustee group, he made an intensive study of inbreeding in loblolly pine, another important species of southern pine. Besides providing basic information on natural inbreeding and its effects, the study revealed a number of mutants. These mutants, along with others recently discovered in slash pine, are currently being used to estimate inbreeding in seed orchards as well as pollen contamination from outside sources. Furthermore, some of the mutants such as "witches' broom dwarfs" and "virescent foliage" have potential as ornamental trees. These are being preserved and are made available to interested research organizations, among them the Ornamental Horticulture Department of the University of Florida.

DEVELOPING MULTIPURPOSE STRAINS

IN the decades since World War II, forest management in the South has been greatly intensified in order to supply wood to the numerous pulp and paper mills and veneer plants which have sprung up in the area. Massive planting programs have been required to improve wild stands, replace harvested stands, and convert unproductive farmland to tree farms. During this period, the production of naval stores has continued to decline and the demand for chemical byproducts of the pulping process has increased. Consequently, forest owners in the naval stores belt are increasingly interested in the timber value of their pine stands as well as high gum yields. Many of them lease trees to naval stores operators before harvest, after which the trees are used for pulpwood and other timber products. Awareness of these factors has led to efforts by Olustee scientists to breed multipurpose strains of slash pine combining high gum yield with such traits as rapid growth, improved quality and yield of wood, and disease resistance.

A concerted effort to develop such superior strains was initiated in the 1960's. Several basic studies of variation and inheritance were established at that time, and these led to the final approach decided upon. Among the important biological facts discovered were the following:

- High-gum-yielding trees occur more frequently than previously believed. Approximately 10 percent of the trees in a natural population will produce at least 50 percent more gum than the average, after the effects of tree size are taken into account, and about 4 percent will produce at least 100 percent more gum.
- Gum-yielding ability and growth rate are genetically correlated. Progenies of high yielders are estimated to contain about 12 percent more stem volume on the average than will progenies of low yielders. The phenotypic correlation (which is based on the demonstrable characteristics of individuals) between these two traits was well-known—large trees tend to produce more gum than do small ones. But revelation of the genetic correlation was both surprising and fortunate.
- No appreciable genetic correlation exists between gum-yielding ability and stem straightness or crown characteristics. Contrary to previous belief, trees that are genetically superior in gum yield occur with equal frequency in both large- and small-crowned trees.

When these findings were revealed, the Olustee tree breeders conceived a practical method of producing multipurpose strains. The method involved capitalizing on the past efforts of several tree improvement workers in the South. In the early 1950's, a number of pulpwood companies had initiated programs designed to obtain better seed for increased growth rate and timber quality. The Georgia Forestry Commission, the Florida Forest Service, and the National Forest Administration had also made similar selections. Many of these organizations had worked closely with geneticists at the University of Florida. Over 1,000 slash pines had been selected for superiority in growth rate, stem straightness, and wood density. Gum yield was not considered in selection.

Here, then, was an unusual opportunity for the Olustee tree breeders. According to the basic patterns of variation and inheritance, the numerous selections made for superiority in traits other than high gum yield should contain some high yielders. Cooperation, plus a little work, was all that was needed to reveal them.

The Olustee scientists launched the program in 1965. The cooperation of 16 organizations having slash pine selections was sought, and permission to evaluate the gum-yielding ability of their selections was requested. Agreement to furnish scions (branches or buds suitable or grafting), pollen, and seed of the selections chosen for the program was also sought. In return, the Forest Service agreed to furnish the cooperators all information obtained in both phenotypic and genetic evaluation of their selections and to supply them with a nucleus of scion material from superior strains developed in the program. Fourteen of the organizations signed cooperative agreements, and even the other two agreed to let their selections be used.

During the subsequent years, the work progressed rapidly under the direction of Charles R. Gansel. Vegetative propagules of the selections have been evaluated for gum-yielding ability, and, as expected, over 100 of them were found to be at least 50 percent superior in this trait. These have been multiplied vegetatively and established in a protected area for future use, and the accepted selections are being progeny tested. Results of the tests conducted to date are favorable—the breeders estimate that if the best one-third of the 100 selections are chosen for use in seed orchards on the basis of progeny tests, genetic gains will be at least 50 percent in gum yield and 25 percent in volume growth. Appreciable gains in stem straightness, crown form, and extractive yield are also expected .

Within 2 to 3 years, selected groups of clones will be made available to cooperators for establishing clonal orchards. In so doing, the breeders plan to develop selection indices in which different weights are assigned to the various traits evaluated. Because some organizations place differing emphases on the various traits according to the product utilized, the selection indices will designate groups of clones tailored to fit the needs of each organization.

Seed from these multipurpose strains will be available in commercial quantities in about 10 years. These strains, supplemented by the high-yielding strain which is already available and which also provides substantial increases in pulpwood and other

wood products as well as gum, have the potential not only of revitalizing the naval stores industry but also of helping meet the ever-increasing demands of the wood industries of the South and Southeast.

CONCLUSION

THE breeding program at Olustee has essentially been one of applied research, with basic research conducted as the need arose. Throughout, the main emphasis has been upon increasing the yield of gum and wood products from slash pine. Success has resulted, in large part, through the application of simple methods of tree breeding, as opposed to the complex methods which have been necessary in the development of such products as superior strains of grain. Although 20 years of research were necessary before breeders in Mexico were able to treble and quadruple the yield of wheat—a span of perhaps 40 generations in the life cycle of the plant—tree breeders at Olustee were able to double the gum yield of a slash pine in only one generation of the tree. These strides were possible because the yielding ability of wild stock in the pine forests was so variable. Although trees mature slowly, great gains could be accrued from small genetic manipulation. Future improvement will, of necessity, involve more advanced methods of breeding.

Of paramount importance to the past success of the program has been the involvement of State and industrial cooperators. Early work by the Forest Service at Olustee and Macon, Ga., for example, was instrumental in arousing the interest of the State experimental stations in research concerning the breeding of forest trees. It is through such cooperative efforts by Federal, State, and private organizations that the program of tree improvement has been advanced and the resurgence of the timber-based economy of the South and Southeast has been effected. If current predictions are correct on the Nation's wood-products need by the year 2000, this region will be called upon to double its current production. In order to do so, these cooperative efforts must be continued and even intensified.

THE FUNCTION OF AN EXECUTIVE

AS nearly everyone knows, an executive has practically nothing to do except to decide what is to be done; to tell somebody to do it, to listen to reasons why it should not be done, why it should be done by someone else, or why it should be done in a different way; to follow up to see if the thing has been done; to discover that it has not; to inquire why; to listen to excuses from the person who should have done it; to follow up again to see if the thing has been done, only to discover that it has been done incorrectly; to point out how it should have been done; to conclude that as long as it has been done, it may as well be left where it is; to wonder if it is not time to get rid of a person who cannot do a thing right; to reflect that he probably has a wife and a large family, and that certainly any successor would be just as bad, and maybe worse; to consider how much simpler and better the thing would have been done if one had done it oneself in the first place; to reflect sadly that one could have done it right in 20 minutes, and, as things turned out, one has had to spend 2 days to find out why it has taken 3 weeks for somebody else to do it wrong.

—Anonymous

SCIENCE NOTES

A COUNT OF VETERINARIANS

THE Soviet Union has more veterinary surgeons than any other country—70,000 compared with 23,000 in the United States, 20,000 in Japan, and 5,500 in Great Britain.

Details of the numbers of veterinarians and domestic animals throughout the world are recorded in an article appearing in a recently issued copy of Hoechst's The Blue Book for the Veterinary Profession.

For convenience the animals are divided into livestock units (LU) whereby one horse equals one LU as do 12 sheep or 5 llamas. Ten camels are the equivalent of 11 LU.

There are between 1,500 and 2,000 LU per veterinarian in the U.S.S.R. while the ratio in Great Britain is about 2,600 and in the United States between 5,000 and 15,000. As a continent, Africa is the poorest in veterinarians with a total of just over 3,000. Even so the ratios of LU vary considerably—from under 10,000 in Egypt to over a million per veterinarian in Guinea.—*The Veterinary Record,* Journal of the British Veterinary Association, August 26, 1972.

The giant fossil egg of the largest bird ever known, Madagascar's elephant bird, extinct for centuries, is estimated to have weighed about 20 pounds, equal to 160 hen eggs.

STORING FOOD UNDERWATER

JAPANESE scientists have found that wheat, corn, barley, rice, soybeans, sesame seed, red beans, et cetera, keep well underwater. Placed in plastic bags filled under carbon dioxide and suspended in a lake for a year and more the foods showed less deterioration and loss than in conventional storage. Construction and maintenance costs were reported to be lower than those of conventional systems. Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

In 1 hour, a modern combine can harvest enough wheat to produce enough bread for 220,000 sandwiches.

"SHOTS" FOR TREES

A method of injecting the chemical benomyl directly into a tree, rather than through the soil, brings much closer the possibility of controlling such destructive tree diseases as Dutch elm, oak wilt, and a wilt of maple. A method of making the fungicide more soluble also was developed.

The research was carried out at the Northeastern Forest Experiment Station Laboratory at Delaware, Ohio. The method has economic and physical advantages. It also could largely avoid the environmental pollution hazards associated with other methods of chemical application.

Botanists in Canada have grown arctic lupines plants from seeds that are believed to have lain dormant for at least 10,000 years in a rodent's burrow in the frozen soil of the Yukon Territory.

"MILK INTOLERANCE"

It is highly inappropriate. . . . on the basis of present evidence, to discourage milk consumption in either the United States or foreign countries because of the fear of milk intolerance."

So concludes the Food and Nutrition Board of the National Research Council, in a statement prepared by its International Nutrition Committee.

Noting recent publicity, "some of it alarmist and sensational", about "milk tolerance" in older children and adults in some non-Caucasian races, the board's report says that "the only basis for most of these public statements is extrapolation from the study of reactions to experimentally administered doses of lactose." The report goes on to say that these age groups of some races do have a considerably lower tolerance than others for the milk sugar, lactose, because of low intestinal lactase (the enzyme responsible for hydrolysis of lactose) and that there is a need "for further research into the implication of lactose tolerance for nutritional programs that encourage milk consumption in various populations."—*Background Information on Lactose and Milk Intolerance,* National Research Council, May 1972.

FUNGUS VS. MOSQUITO

Clyde Umphlett, head of the botany department of Clemson University, is working with a microscopic fungus that lives in water and has an uncanny ability to track down mosquito larvae and kill them. Umphlett has been studying the pearl-gray fungus, called *Lagenidium,* since 1962, when he discovered it in mosquito larvae taken from a trash dump near Dublin, Ga.

Laboratory tests have shown that the fungus has a kill rate of up to 95 percent among larvae of the southern house mosquito, yellow fever, and several other tropical disease carriers, and the northern and southern salt marsh mosquitoes.

Small-scale field tests to determine how well the fungus performs out of the laboratory are scheduled at the Center for Disease Control Laboratory, Savannah, Ga. Biochemical and physiological studies are being carried out simultaneously by research partners at the University of North Carolina at Chapel Hill.

If the experiments are successful, this form of biological warfare could reduce the need for spraying mosquito breeding grounds with chemical insecticides or oil.—*BioScience,* American Institute of Biological Sciences, July 1972.

There is hardly an area of everyday life which has not been and is not being improved as the result of agricultural research. The returns to the consuming public far exceed the costs of this research, and they far exceed returns to production agriculture.—Allan Grant, president of the California Farm Bureau, in California Farm Bureau Monthly, September 1972.

MERCURY POISONING IN FOWL

In a study of mercury poisoning in commercial broiler-type chickens, ducks, and pheasants, ducks had the highest mercury levels in their tissues, and chickens the lowest.

The tests, conducted at the Canada Agriculture Research Station at Lethbridge, Alberta, showed that ducks also retained more mercury in their tissues over a period of time after they had been put on a mercury-free diet. Chickens retained the least.

The birds were all fed four levels (0.0, 0.33, 3.3 and 33.0 parts per million) of mercury added to their diets which were based on wheat and soybean meal. After 35 days, body weight was lower in all three species on the 33 p.p.m. diet than on the other three diets. Mortality with the 33 p.p.m. diet was 90 percent for pheasants, 85 percent for ducks, and 7.5 percent for chickens.

It is not know why chickens tolerate higher levels of mercury than do ducks and pheasants, but it could be due to any or all of the following: The lower feed-to-grain ratio of chickens, which would result in less total mercury consumed per unit weight; difference in mercury absorption rates between the three species; and difference in mercury excretion rates between the three species.

The results stress the importance of preventing even small amounts of mercury from being fed to any of the three species used in the study.—*Canadian Journal of Animal Science,* June 1972.

FORUM

FARM EFFICIENCY AND RESEARCH

THE long-felt view among agricultural researchers is that research to increase agricultural production and increase efficiency is good, that more research would be better, and that the largest possible amount of research would be the best possible thing. In other words, the functional relationship between the amount of research and the general well-being of the country is assumed to be positive, linear, and steep.

But when the total adjustments that flow from research are taken into account, this may not be the case. It may be that the true relationship between the amount of research and the general well-being of the country is in the form of a curve. It may be that there is some optimum amount of research that will maximize the good effects, that to do less than that would be to forego potential benefits, and that to do more would subject farmers—and some nonfarmers—to a stress of adjustment that is greater than they can readily bear. In that case, the functional relationship would be described in the form of a curve, rising steeply at first, reaching a maximum at some point, then turning down and at some point becoming negative.

In general, physical and biological scientists think the function is linear and social scientists think it is a curve. I count myself among the latter.

This article is from an address by the author, Don Paarlberg, before the 21st annual meeting of the Agricultural Research Institute, National Academy of Sciences Auditorium, Washington, D.C., Oct. 10, 1972.

If it is a curve, the next question is, what is our position on the curve? I think, and this has to be a matter of subjective judgment, that we are somewhere near the apogee of the curve. It is not clear to me, for example, that rapid mechanization of tobacco production, *all things considered,* would be beneficial to farm people or to the country.

Clearly, if we do helpful research that facilitates the adjustment process, the amount of physical and biological research we can assimilate is increased.

There is a prevailing belief that farmers should capture for themselves the total gain that comes from increased agricultural efficiency. But this is not the way it works. In a truly competitive system, no industry can capture for itself the gains that result from increased efficiency in that industry. These gains are disseminated generally throughout the economy. For example, there have been enormous gains in the efficiency of making nitrogen fertilizer during the past 10 years. But the fertilizer industry did not capture all of these gains. Much of the gain was passed on to the farmers who bought the fertilizer, and then on to the consumers who bought the lower-priced food. The real price of fertilizer fell 7 percent during the past decade. The farm price of anhydrous ammonia fell 50 percent from $150 to $75 per ton despite general price inflation.

The barber has made virtually no technical gains for about 30 years, since the electric clipper was invented. The manual laborer, digging a ditch, has made no technical gains since the invention of the spade. Yet their real returns have risen. They have shared in the general efficiency gains experienced by the entire society.

Farmers have experienced increased incomes, over time, not just as a result of efficiency gains in agriculture but as members of society. Let me explain.

We tend to think of the economy as if it were a plastic ice cube tray, with the various sections retaining the water poured into them. Actually the economy is much more like a tray with a loose removable divider, so that water seeps through the cracks and finds its level, irrespective of where it was first poured.

How can gains be won by those who have done nothing to earn them? Because, over time, workers are mobile. They move from less-rewarding to the more-rewarding activities. Thus they manage to

equate, with lags and in a rough way, the supplies of and demands for labor in various vocational fields at something like normal relationships. The efficiency gains achieved in various sectors are dispersed throughout. The impact of adversity is softened by an economy that is humanitarian in effect if not in intent. The fact that some people make the adjustment lessens the need for others to adjust.

The competitive society ingeniously—and in my judgment, appropriately—thwarts those who would hold their gains closely to themselves and alleviates the problems of those in stagnant or declining industries. In the long run we advance more as members of the total society than we do as individual persons or as vocational groups.

I do not think agricultural researchers should be disheartened with the fact that the results of their work are widely disseminated throughout the economy. On the contrary, they should be happy that so many people share in the benefits of their work. This is the appropriate reason for the use of public revenue in support of agricultural research. My concluding remark is that it is inappropriate, therefore, in the accounting process, to charge agriculture with the whole cost of agricultural research. The primary beneficiaries are the consumers, all of us, not just the farmers.

Don Paarlberg,
Director of Agricultural Economics,
U.S. Department of Agriculture,
Washington, D.C.

THE AUTHORS

DONALD K. BARNES ("Evolution of Hybrid Alfalfa") is a research geneticist, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, St. Paul, Minn. He holds B.S. and M.S. degrees from the University of Minnesota and a Ph. D. from Pennsylvania State University. Dr. Barnes assumed his present assignment at the University of Minnesota in 1969. He has held research positions with the USDA at the U.S. Regional Pasture Research Laboratory, University Park, Pa.; U.S. Federal Experiment Station, Mayaguez, P.R.; and Plant Industry Station, Beltsville, Md. Dr. Barnes' special interests include breeding for disease and insect resistance, determining basic inheritance patterns for alfalfa traits, and developing pollen controls.

WALTER R. CHILDERS ("Evolution of Hybrid Alfalfa") is a plant breeder, Canada Department of Agriculture, Research Branch, Research Station at Ottawa, Canada. He holds a B.Sc. degree from McGill University, Montreal and M.S. and Ph. D. degrees from the University of Wisconsin. Dr. Childers is doing research on grass breeding along with his research in male sterility in alfalfa. Dr. Childers' special field is grass breeding where he has licensed five varieties which have had successful utilization in forage production.

KEITH W. DORMAN ("Breeding Slash Pine In Florida: A Success Story") is principal silviculturist, Division of Planning and Application, Southeastern Forest Experiment Station, USDA Forest Service, Asheville, N.C. He received a B.S. degree from Iowa State University, and joined the Forest Service that year. At various times he has been in charge of tree breeding work at Olustee, Fla., Macon, Ga., and Asheville, N.C.

GEORGE F. FRIES ("PCB Residues: Their Significance to Animal Agriculture") is a research animal scientist, ARS, U.S. Department of Agriculture. A graduate of Washington State University, he received an M.S. from the University of Kentucky and Ph. D. from Michigan State University. Dr. Fries joined the Animal Husbandry Research Division, ARS, in 1959 and conducted research on nutritional evaluation of forages at Bozeman, Mont. After a brief tour in Ithaca, N.Y., he transferred to Beltsville in 1962 where he conducted studies on radioactive fallout. Since 1966 his primary research interests have been the metabolism of pesticides and related industrial chemicals by farm animals. Dr. Fries is a member of the ARS's Pesticides Degradation Laboratory, Agricultural Environmental Quality Institute.

RALPH E. McNEES ("Breeding Slash Pine In Florida: A Success Story") is writer-editor, Southeastern Forest Experiment Station, USDA Forest Service, Asheville, N.C. He received a B.A. degree from Arkansas State University and joined the Forest Service in 1967. He is presently working toward an M.A. degree at Western Carolina University under the Government Employees Training Act.

A. E. SQUILLACE ("Breeding Slash Pine In Florida: A Success Story") is chief plant geneticist, Work Unit, Breeding Naval Stores Pines, Naval Stores and Timber Production Laboratory, Southeastern Forest Experiment Station, USDA Forest Service, Olustee, Fla. He received a B.S. degree from the University of Minnesota, an M.S. degree from the University of Montana, and a Ph.D. degree from the University of Florida. Most of his 28 years with the Forest Service have been spent in the Northwest breeding western white pines resistant to blister rust and in the Southeast breeding high-gum-yielding slash pines.

T.C. TSO ("The Potential for Producing Safer Cigarette Tobacco") is leader, Tobacco Laboratory, Plant Genetics and Germplasm Institute, NARC, ARS, U.S. Department of Agriculture, Beltsville, Md. He received B.S. and M.S. degrees from the University of Nanking, China, and Ph. D. from Pennsylvania State University. Dr. Tso has been active in tobacco research since 1941 at various locations and capacities. He joined the tobacco production research in the USDA in 1952. He became leader of the tobacco quality investigations in 1967, and was appointed to his current position in 1972. Dr. Tso is a member of the Tobacco Working Group, Lung Cancer Task Force, NCI; and also a member of the International Tobacco Working Group.

U.S. GOVERNMENT PRINTING OFFICE : 1972 O - 479-943

AGRICULTURAL SCIENCE REVIEW is a critical review journal designed to provide a common point of understanding among agricultural scientists by publishing authoritative commentary on the current state of agricultural research. *Review* does not publish primary research reports. Yearly subscription rate is $2.00, domestic; $2.50, foreign. Single copies are 75 cents. Subscription orders should be sent to the Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.